

Preparation of Environmental Documentation and Risk Assessments for the USDA/Forest Service

Prepared by:
Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950

E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com



January 21, 2007

REVISION NOTE

This is a modification to SERA MD 2006-01a, Preparation of Environmental Documentation and Risk Assessments for the USDA/Forest Service, dated March 3, 2006. A minor change involves a brief note on the addition of an exposure scenario for a small mammal consuming contaminated grass (Section 4.2.2.3). Based on comments from peer-reviewers, this scenario was added to Forest Service risk assessments during 2006. A more substantial modification has been made to Section 4.4 (Risk Characterization for Ecological Effects). This modification involves a much fuller and more explicit discussion of the differences between the U.S. EPA approach to risk characterization and the approach taken in Forest Service risk assessments.

WAIVER OF CONFIDENTIALITY

No part of this document is claimed as confidential. To the contrary, the purpose of this document is to disclose how SERA, Inc. conducts risk assessments for the USDA/Forest Service and related organizations. The government, general public, and other interested parties should and must have full access to this information.

SERA Inc. will be grateful for any written comments or criticisms of the methods detailed in this report. Well-documented and detailed suggestions for improving these methods are most welcome.

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1. INTRODUCTION

1.1. SCOPE AND INTENT

SERA, Inc. has prepared risk assessments for the USDA Forest Service, Office of Forest Health, since 1995 [USDA/FS Contract No. 53-3187-5-12]. In addition, SERA, Inc. has prepared various other risk assessments for both the Forest Service and USDA/APHIS since 1990. During this 15-year period, the methods used to conduct these risk assessments evolved and changed substantially. The purpose of this document is to describe in detail the methods currently used by SERA, Inc. in the conduct of these risk assessments.

The risk assessments prepared by SERA consist of analyses of both human-health effects and ecological effects to support an assessment of the environmental consequences of the use of various chemicals in Forest Service programs. In this context, *support* does not imply that any attempt is made to bias analyses toward making the chemicals look safe. To the contrary, the Forest Service has accepted and often insisted on the use of very conservative methods both in the assessment of exposures as well as consequences. These methods are detailed in the current document.

Although the risk assessments are technical support documents and typically address specialized technical areas, an effort is made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. At the same time, the risk assessments must be sufficiently detailed and technical to allow for review by individuals with substantial and often highly specialized expertise in various areas of environmental science. As a consequence, some of the discussions and calculations presented in each risk assessment may be very complicated. These discussions are presented as necessary in the major sections of each risk assessment. Nonetheless, each of the major sections are preceded by an overview section that is intended to be readily understood by most readers.

The basic philosophy for preparing the risk assessments is that *each risk assessment must be totally transparent*. If a risk assessment is to be properly reviewed, understood, critiqued, and used, the source of all numbers, the calculations used in generating the numbers, and the assumptions used in manipulating the numbers must be outlined clearly. In some respects, the transparency of a risk assessment is more important than the specific methods or calculations used to prepare it. Risk assessment is a form of analysis that relies on scientific method but is not itself a *science*. Reasonable individuals may disagree over which of the numerous methods, tools, and approaches should be used to prepare a risk assessment. Often, available information is not sufficient to support one analytical approach over another. Professional judgment must then be used to select the method; in which case, the risk assessment must clearly state which assumptions are used and why. As long as the assumptions are made clear, the quality of the risk assessment may be reviewed and the risk assessment may be critiqued as appropriate and improved in review.

As part of this transparency, this current document details the methods that are used to prepare risk assessments for the USDA Forest Service. To the extent possible, the organization of this document parallels the organization of the risk assessments. This introductory chapter presents the basic conceptual framework for the risk assessment process, briefly discusses the organization of the risk assessment, and describes the methods used to identify and screen information for inclusion into the risk assessments. The subsequent sections of this document further parallel those of each risk assessment: Program Description (Section 2), Human Health Risk Assessment (Section 3), and Ecological Risk Assessment (Section 4). As with each of the risk assessments, various types of supporting information are included in appendices. The organization of all of these chapters is very similar to the organization of the corresponding chapters in each risk assessment.

1.2. RISK ASSESSMENT FRAMEWORK

1.2.1. Basic (NAS) Approach

In 1983, the National Research Council of the National Academy of Sciences (NRC 1983) recommended a basic approach for risk assessments that are conducted by or for groups within the government. NRC (1983) recommended a four step process: hazard identification, exposure assessment, dose-response assessment, and risk characterization. Each of the two risk assessment chapters (human health and ecological effects) are organized in this manner. As a corollary, the fundamental principle in all SERA risk assessments is that:

Risk cannot be characterized quantitatively unless a hazard can be identified, exposures can be quantitatively estimated, and a dose-response relationship can be expressed quantitatively.

Each of the basic steps are summarized in the following subsections. Details of these steps are given in the appropriate sections of methodology for human health risk assessments (Section 3) and ecological risk assessments (Section 4).

1.2.1.1. Hazard Identification – Hazard identification is the process of identifying what, if any, effects a compound is likely to have on an exposed population. Hazard identification is the first and most critical step in any risk assessment. Unless some plausible biological effect can be demonstrated, the nature of the subsequent dose-response assessment and risk characterization is extremely limited. Both the human health and ecological risk assessments are prepared using *in vivo* and *in vitro* data from experimental animal studies. Additional sources of information like epidemiology studies, case reports, and clinical investigations are used to prepare human health risk assessment. Studies on various model nontarget test species (e.g., ducks, quail, fish, aquatic invertebrates, plants, and terrestrial invertebrates) are commonly available to strengthen an ecological risk assessment. In addition, available field studies on nontarget species are used in ecological risk assessments in much the same way epidemiology studies are used in human health risk assessments. The hazard identification is based on a review of the toxicological and pharmacokinetics data and is arranged to focus on the dose-response and dose-severity

relationships. Of these two relationships, the dose-severity relationship is generally more relevant for non-carcinogenic effects in humans and nontarget species.

The severity scale used to conduct the risk assessment typically employs four levels of severity, which are defined in Table 1-1. The terminology used in human health and ecological risk assessments is somewhat different, but the concepts are virtually identical. In human health risk assessment, severity is typically defined by the consequences of different levels of exposure. These include the no-observed-effect level (NOEL), no-observed-adverse-effect level (NOAEL), adverse-effect level (AEL), and frank-effect level (FEL). An additional term, lowest-observed-adverse-effect level (LOAEL) is sometimes used to designate the lowest AEL. This scale, with minor differences in nomenclature, is used by many government agencies to classify the toxicological effects observed in experimental or epidemiology studies. In the ecotoxicology literature, the term NOEC—no observed effect concentration—is sometimes used rather than the term NOEL. As indicated in Table 1-1, these terms as well as their variations are synonymous.

The hazard identification process involves making judgments about which effects are most relevant to the assessment of human health or nontarget species. During this process, studies may be eliminated from consideration because they are inherently flawed, or because they are grossly inconsistent with the preponderance of other studies.

Although hazard identification results in a qualitative determination, quantitative methods are usually required as in most other assessments of causality. For instance, the process of hazard identification often hinges on a statistical assessment of exposure-response or dose-response relationships. Furthermore, hazard identification must also consider fundamental and qualitative differences among species. Depending on the chemical of concern, hazard identification also may include the use of quantitative or qualitative structure activity relationships or differences in pharmacokinetics.

1.2.1.2. Exposure Assessment – The exposure scenarios considered in a risk assessment involving pesticide exposure are determined by the application method and the chemical and toxicological properties of the compound. Depending on the properties of the chemical and the application method, the risk assessment may consider acute, subchronic, or chronic durations of oral, dermal, inhalation or combined exposure to the pesticide.

1.2.1.2.1. HUMAN HEALTH – Exposure scenarios are developed for workers and members of the general public. For each group, two types of exposure scenarios are generally taken into consideration: *general exposure* and *accidental/incidental exposure*.

The term *general exposure* refers to human exposure resulting from the normal use of the chemical. For workers, general exposure involves the handling and application of the compound. These general exposure scenarios can be interpreted relatively easily and objectively. The exposure estimates are calculated from the amount of the chemical handled/day and the exposure rates for the worker group. Although each of the specific exposure assessments for

workers involves degrees of uncertainty, the exposure estimates are objective in that they are based on empirical relationships of absorbed dose to pesticide use. For the general public the general exposure scenarios are somewhat more arbitrary and may be less plausible. For each pesticide, at least three general exposure scenarios are considered, including walking through a contaminated area shortly after treatment, the consumption of ambient water from a contaminated watershed, and the consumption of contaminated vegetation. These three scenarios are consistently used because one of them usually leads to the highest estimates of exposure. Additional scenarios discussed below may be considered for each of the individual compounds as warranted by the available data and the nature of the program activities.

Some, if not all, of these general exposure scenarios for the general public may seem implausible or at least extremely conservative. For example, in many cases compounds are applied in relatively remote areas and so it is not likely that members of the general public would be exposed to plants shortly after treatment. Similarly, the estimates of longer-term consumption of contaminated water are based on estimated application rates (lbs a.i./acre) and monitoring studies that can be used to relate levels in ambient water to treatment rates in a watershed; however, in most pesticide applications, substantial portions of a watershed are not likely to be treated. Finally, the exposure scenarios based on longer-term consumption of contaminated vegetation assume that an area of edible plants is inadvertently sprayed and that these plants are consumed by an individual over a 90-day period. While such inadvertent contamination might occur, it is extremely unlikely to happen as a result of directed applications (e.g., backpack applications). Even in the case of boom spray operations, the spray is directed at target vegetation and the possibility of inadvertent contamination of cultivated or edible vegetation would be low. In addition, for herbicides and other phytotoxic compounds, it is likely that the contaminated plants would show obvious signs of damage over a relatively short period of time and would therefore not be consumed.

All of the factors discussed above concerning general exposure scenarios for the general public have merit and must be considered in the interpretation of the risk characterization (Section 3.4). Thus, the *typical* hazard to the general public may often be negligible because significant levels of exposure are not likely. For the general public, the general exposures may be regarded as *extreme* in that they are based on very conservative exposure assessments and/or very implausible events. Nonetheless, these general exposure assessments are included because the risk assessment is intended to be extremely conservative with respect to potential effects on the general public, and to provide estimates regarding the likelihood and nature of effects after human exposure to pesticides.

Accidental/incidental exposure scenarios describe specific examples of gross over-exposure associated with mischance or mishandling of a chemical. All of these exposure scenarios are arbitrary in that the nature and duration of the exposure is fixed. For example, the worker exposure scenario involving immersion of the hands is based on a 1-minute period of exposure but could just as easily be based on an exposure period of 5 seconds or 5 minutes. Similarly, the consequences of wearing contaminated gloves could be evaluated at 4 hours rather than at 1 hour.

These scenarios are intended to provide an indication of relative hazard among different pesticides and different events in a manner that facilitates conversion or extrapolation to other exposure conditions.

Like the general exposure scenarios, the accidental exposures for the general public may be regarded as more extreme than those for workers. Three scenarios are included in each exposure assessment. They include direct spray, the consumption of contaminated water shortly after a spill, and the consumption of contaminated vegetation shortly after treatment. The direct spray scenario is clearly extreme. It assumes that a naked child is sprayed directly with a pesticide as it is being applied and that no steps are taken to remove the pesticide from the child for 1 hour. There are no reports of such incidents in the literature, and the likelihood of such an incident occurring appears to be remote. Nonetheless, this scenario and others like it are useful not only as a uniform comparison among pesticides but also as a simplifying step in the risk assessment. If the '*naked child*' scenario indicates no basis for concern, other dermal spray scenarios will not suggest a potential hazard and need not be explored. If there is a potential hazard, other more plausible exposure scenarios may need to be considered. The other two accidental scenarios are similarly intended to serve as uniform comparisons among chemicals as well as a means of evaluating the need to explore additional exposure scenarios.

In all cases, the level of exposure is directly proportional to the exposure parameters. The exposure associated with wearing gloves for 4 hours is 4 times the exposure associated with wearing contaminated gloves for 1 hour. Similarly, the general exposure scenarios for workers are based on an 8-hour work day. If a 4-hour application period were used, the hazard indices would be reduced by a factor of two. As another example, general exposure scenarios for both workers and the general public are linearly related to the application rate. Consequently, if the application rate were to double or vary by some other factor, the estimated exposure would double or vary by the same factor. Thus, the specific exposure parameters used in the risk assessment are selected to allow for relatively simple extrapolation to greater or lesser degrees of exposure.

Additional variability is taken into consideration by estimating exposure doses or absorbed doses for individuals of different age groups (i.e., adults, young children, toddlers, and infants). Children may behave in ways that increase their exposure to applied pesticides (e.g., long periods of outdoor play, pica, or imprudent consumption of contaminated media or materials). In addition, anatomical and physiological factors, such as body surface area, and breathing rates and consumption rates for food and water, are not linearly related to body weight and age. Consequently, the models used to estimate the exposure dose (e.g., mg/kg body weight/day) based on chemical concentrations in environmental media (e.g., ppm in air, water, or food) indicate that children, compared with individuals of different age groups, are generally exposed to the highest doses of chemicals for a given environmental concentration.

1.2.1.2.2. ECOLOGICAL EFFECTS – The exposure assessments for ecological effects are conceptually similar to those conducted in the human health risk assessment, and for many

terrestrial organisms the exposure assessments are parallel to those used in the human health risk assessment. Similarly, exposures of aquatic species are typically based on the same estimates of concentrations of the chemical in water that are used in the human health risk assessment.

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. Estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg body weight. For dermal exposure, the units of measure usually are expressed in mg of agent per cm² of surface area of the organism and abbreviated as mg/cm². In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm² and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

For the exposure assessments discussed below, general allometric relationships are used to model exposure (e.g., Boxenbaum and D'Souza 1990). These relationships dictate that for a fixed level of exposure (e.g., concentrations of a chemical in food or water), small animals will receive a higher dose, in terms of mg/kg body weight, than large animals will receive.

Based on allometric relationships, it would be possible to model exposure in a very large number of nontarget terrestrial animals. This approach has been used in some past USDA assessments. This approach is no longer used because highly species-specific exposure assessments are of little use in the absence of species-specific dose-response assessments. Thus, if the pesticide-specific information indicates that large mammals may be more sensitive than smaller mammals (i.e., in contrast to the more general relationship noted above), both large and small mammals are modeled separately. Similarly, if the available information suggests that the compound under review may be more toxic to birds than to mammals, separate exposure assessments are conducted for both birds (large and small) and mammals. The basic philosophy behind this approach is that the exposure assessment should not be more complicated than the dose-response assessment.

Generic estimates of exposure are always given for a small mammal. A body weight of 20 g is used for a small mammal, which approximates the body weight of small mammals like mice, voles, shrews, and bats. Other body weights, food consumption, and caloric requirements for mammals and birds are taken from U.S. EPA (1993). The computational details for each exposure assessment presented in this section are provided in standard worksheets (see Appendix 3). Depending on the available toxicity data and the uses of the chemical under review, exposure assessments may be made for larger mammals, birds, various terrestrial invertebrates, and terrestrial plants. The specific scenarios most often considered are detailed in Section 4.2.

1.2.1.3. Dose-Response Assessment – The purpose of the dose-response assessment is to describe the degree or severity of risk as a function of dose. In classical toxicology, dose-response assessments are usually expressed as linear or non-linear equations, such as probit analysis and the multistage model, respectively. Using these methods, the prevalence or magnitude of a response can be estimated for any dose level. In regulatory toxicology, this approach is the exception rather than the rule.

Most dose-response assessments in regulatory toxicology, as discussed below, result in point estimates. Although some methods in regulatory toxicology use dose-response models, the regulatory value used is a point estimate. For example, U.S. EPA cancer risk assessments usually employ a form of the multistage model or some other linear dose-response relationship that provide measures of variability or error. The estimate used in setting exposure criteria, however, is typically a point estimate that is a single value rather than a range of values. The results of other commonly used dose-response assessments, such as RfDs, and RfCs, are point estimates of doses that are not believed to be associated with any adverse effect and that are not directly related to a dose-response model.

The practice of relying on point estimates in regulatory toxicology is grounded in the history of this discipline (Dourson and Stara 1983). From its inception, the focus of regulatory toxicology has been the development of criteria (i.e., levels of exposure that are defined as *safe*). Consequently, the methods used in regulatory toxicology are conservative.

Consistent with the recommendation of NRC (1983) that various groups within the federal government adopt common risk assessment methodologies, standard dose-response assessments are generally based on reference values, like RfDs, derived by other government agencies. This approach avoids a duplication of effort, capitalizes on the expertise of other organizations, and decreases the size, complexity, and cost of risk assessments.

In cases for which these standard approaches yield evidence of potential risk, other statistical methods such as categorical regression may be used to characterize the likelihood and severity of the risk. Categorical regression analysis is used as a tool to supplement RfDs and analogous values. The method defines a relationship between responses that can be categorized according to exposure dose and duration (factors that may influence the response), and estimates the probability that a group of animals subjected to a given exposure will be classified into a particular category (Dourson et al. 1997, Durkin et al. 1992, Guth et al. 1997). Categorical regression as well as other methods (quantitative and semi-quantitative) are discussed further in Section 3.3.5.

In most respects, dose-response assessments for ecological effects are conceptually similar to the methods employed in the human health risk assessments, with one major exception. Human health risk assessments focus on protecting the individual. This is why uncertainty factors (sometimes very large) are used to derive RfD values and why cancer risk is estimated using very conservative assumptions. In ecological risk assessment, the focus is on a population or

community rather than an individual. Thus, the use of uncertainty factors is less common and the general methods for dose-response assessment are less conservative.

For terrestrial mammals, the dose-response assessment generally is based on the same data used to derive the RfD in the human health risk assessment: an NOAEL from a chronic exposure study. The data on other terrestrial animals, both birds and invertebrates, are often not as detailed as the available information on experimental mammals. Fewer toxicological endpoints are examined, and, at least for vertebrates, lifetime or chronic studies are seldom available.

For some terrestrial plants as well as some aquatic species, sensitive life-stage studies are often available. Such studies include egg-and-fry studies in fish, life-cycle toxicity studies in *Daphnia magna*, and seed germination and growth studies in plants, all of which are required by the U.S. EPA for the registration of herbicides. The studies are obtained and assessed following the same criteria applied to studies for the human health risk assessment. The principal difference is that NOEL, NOEC, or LD or LC values are used directly rather than RfD values that involve the application of uncertainty factors.

Nonetheless, dose-response assessments for some nontarget species considered in a risk assessment can be complicated (Section 4.3). As in the human health dose-response assessment, the nature of the available data as well as the potential risk may dictate the use of relatively complex dose-response analyses.

1.2.1.4. Risk Characterization – Conceptually, risk characterization is simply the process of comparing the exposure assessment to the dose-response assessment. In this process, risk is characterized quantitatively either as a ratio or as an incidence of response or a defined risk level – i.e., a risk of 5%.

Because the risk characterization flows directly from the exposure and dose-response assessments, the complexity and clarity of the risk characterization will be dependent on complexity and clarity of both the exposure and dose-response assessments. In most cases, risk will be quantitatively characterized as a ratio: a level of exposure divided by some defined effect level. In the human health risk assessment, the defined effect level is almost always the reference dose (RfD), and the ratio of the exposure to the reference dose is referred to as the hazard quotient (HQ). In the ecological risk assessments, the defined effect level is may be an NOEC or a risk level. The risk level, in turn, may be a lethal dose (e.g., LD₅₀ or some other response level such as an LD₂₅) or a dose causing some risk of a non-lethal effect (e.g., an ED₅₀ or ED₂₅). For aquatic organisms and for some terrestrial organisms for which exposure is characterized by a concentration rather than a dose, the defined risk levels may be expressed as a lethal concentration (LC₅₀ or some other response level) or a sublethal concentration that leads to some effect (e.g., an EC₅₀). In general, the Forest Service prefers to use NOAEL or NOEC values in risk characterizations. If NOAEL or NOEC values are not available, a sublethal effective dose at some response rate (e.g., ED_x or EC_x where *X* is some level of response) is generally preferred

over a lethal response rate (e.g., LD_x or LC_x). While these ratios are sometimes referred to as HQs, more suitable terms are risk quotients (RQs).

If sufficient data are available and if the simple HQs or RQs suggest some level of concern, dose-response or dose-severity relationships may be used to characterize risk. Dose-response relationships most often involve explicit dose-response functions that lead to an explicit estimate of risk (e.g., a response rate of 13.2% for some effect or an 8% decrease in some biological function). Dose-severity relationships are typically less quantitative and lead to some assessment of what effects might be observed in a population at various levels of exposure. A fuller discussion of the quotient methods (HQs and RQs) as well as the dose-response and dose-severity relationships are given in Section 3.4 (Human Health Effects) and Section 4.4 (Ecological Effects).

1.2.2. Elaborations

1.2.2.1. Probabilistic Risk Assessment – *Variability* and *uncertainty* may be dominant factors in any risk assessment, and these factors should be expressed. Within the context of a risk assessment, the terms *variability* and *uncertainty* signify different conditions. In general, *variability* and *uncertainty* can be distinguished from each other depending on the state of knowledge or information. *Variability* reflects the knowledge of how things may change. By acquiring more knowledge or information, better estimates of variability may be obtained but the *variability* itself will not decrease – i.e., it is inherent in the population or system being considered. Differences in human body weights are a good example of variability. Uncertainty reflects a lack of knowledge and uncertainty can be reduced by acquiring information. For example, while the toxicity of herbicides has been tested in the honey bee, very little information is available on the toxicity of most herbicides to other nontarget terrestrial insects. This leads to uncertainty (in terms of how representative the honey bee is for other insects) but this uncertainty can be reduced by conducting experiments on the toxicity of the herbicide to other insects.

Variability may take several forms. For this risk assessment, three types of variability are distinguished: *statistical*, *situational*, and *arbitrary*. *Statistical variability* reflects apparently random patterns in data. For example, various types of estimates used in this risk assessment involve relationships of certain physical properties to certain biological properties. In such cases, best or maximum likelihood estimates can be calculated, as well as upper and lower confidence intervals that reflect the statistical variability in the relationships. *Situational variability* describes variations depending on known circumstances. For example, the application rate or the applied concentration of an herbicide will vary according to local conditions and goals. As discussed in the following section, the limits on this variability are known and there is some information to indicate what the variations are. In other words, *situational variability* is not random. *Arbitrary variability*, as the name implies, represents an attempt to describe changes that cannot be characterized statistically or by a given set of conditions that cannot be well defined. This type of variability dominates some spill scenarios involving either a spill of a chemical on to the surface of the skin or a spill of a chemical into water. In either case, exposure

depends on the amount of chemical spilled and the area of skin or volume of water that is contaminated.

In order to quantitatively address both variability and uncertainty, risk assessment methods generically referred to as ***probabilistic risk assessment*** have been and continue to be developed. The general approach for probabilistic risk assessment, particularly with respect to ecological species, has been articulated by Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM 1999). The basic approach given in ECOFRAM (1999) involves a tiered risk assessment process:

- Tier 1: Very conservative screening methods involving worse case assumptions in terms of both exposure and dose-response. Risk is typically expressed as a point estimate such as an HQ or RQ.
- Tier 2: Typically elaborates or refines the exposure assessment to include more realistic estimates of exposures and may elaborate the dose-response assessment to include the use of full dose-response curves. Risk may be expressed in terms of probabilities rather than point estimates.
- Tier 3: An extension of a Tier 2 approach that may involve the inclusion of data on additional species (e.g., species sensitivity distributions) and more sophisticated exposure models.
- Tier 4: Is the most complex risk assessment and may involve experimental or monitoring programs designed to definitively characterize either exposure and toxicity and the use of all available data including microcosm, mesocosm, and field studies.

As implied by the term *Tier*, probabilistic risk assessments under the general ECOFRAM model are designed to be conducted in stages going from the most conservative or worst-case approach (Tier 1) to less extreme and presumably more realistic assessments. Because this staged approach typically results in progressively lessened perceptions of risk, probabilistic risk assessments have been criticized as simply mechanisms to make risk disappear by mathematical manipulations. This criticism is addressed in ECOFRAM (1999) and is largely unfounded. While any risk assessment, probabilistic or otherwise, can be manipulated to distort risk (either upward or downward), the proper application of probabilistic risk assessment typically results not in conflicting risk characterizations at the different tiers but rather in more fully elaborated and refined risk assessments.

The nomenclature of probabilistic risk assessments, particularly as embodied in ECOFRAM (1999) is somewhat different from that of NAS (1983) but the concepts are essentially the same. The first stage of a probabilistic risk assessment is typically referred to as the *Problem Formulation*. This is similar to the *Hazard Identification* as defined by NAS (1983) but focuses on identifying which organisms are likely to be at greatest risk. The other stages of the risk

assessment process defined by ECOFRAM (1999) are exposure characterization, effects characterization, and risk characterization and correspond closely to more general definitions given by NAS (1983) for the exposure assessment, dose-response assessment, and risk characterization.

In the higher tiered risk assessments, the probabilistic approach is based on more sophisticated methods of handling data and expressing both variability and uncertainty. A central feature of many higher tiered probabilistic risk assessments is Monte Carlo Analysis. *Monte Carlo Analysis* is a general term for any simulation that uses probability distributions rather than point estimates to represent and approximate the variability in a system model. The method was originally developed in the 1940's, shortly after the development of computers, to make probabilistic approximations to the solutions of mathematical equations or models that could not be solved analytically (U.S. EPA/Risk Assessment Forum, 1997).

Monte Carlo Analyses can be relatively simple or very complicated depending on the simplicity or complexity of the model. As a simple example, take a situation in which we knew that a population of individuals will be exposed each day to up to 200 mg of a chemical. In this population, the smallest individual will have a body weight of about 52 kg. Thus, the maximum daily dose is about 3.8 mg/kg body weight. In addition, we knew that the RfD for the general population is 3.5 mg/kg. Taking a standard ratio approach using point estimates (Section 1.2.1.4), the hazard quotient would be about 1.1, somewhat above the level of concern. This would be a standard point-estimate worst-case approach and the risk assessment would conclude that some unspecified number of individuals could be subject to exposures that would not be generally considered acceptable.

Suppose, however, that the average body weight was 70 kg and the body weights in the population evidenced a normal distribution with a standard deviation of 10 kg. In addition, suppose that we knew that not all individuals would be exposed to the same amount of the chemical but that the amount could vary from 50 mg/day to 200 mg/day. Lastly, while the RfD was 3.5 mg/kg/day, we also knew that some individuals could be more sensitive and might respond with an adverse effect at a dose above 2 mg/kg/day, but that other individuals would not respond adversely until the dose reached 10 mg/kg/day. This sort of variability could be modeled in a Monte Carlo Analysis with the following assumptions:

Parameter	Distribution
Body weight	Normal distribution with a mean of 70 kg and a standard deviation of 10 kg
Exposure	Uniform distribution with a range of 50 mg/day to 200 mg/day.
RfD	Triangular with a mode of 3.5 mg/kg/day, a lower limit of 2 mg/kg/day and an upper limit of 10 mg/kg/day

An illustration of the results of a Monte Carlo Analysis of this simple model is given in Figure 1-2. Under the conditions of the simulation, the hazard quotient would be greater than unity (the level of concern for this scenario) for about 5% of the population.

Note that the use of a Monte Carlo simulation does not necessarily change the conclusions risk assessment. In the above example, the simulation is consistent with the worst-case point estimate approach: some people will be at risk. The Monte Carlo simulation, however, does incorporate more information into the assessment and allows the risk assessor to better characterize the consequences – i.e., about 5% of the individuals may be exposed to more of the agent than would be generally considered acceptable.

Most practical Monte Carlo simulations are much more complicated and may involve quantitative considerations of differences in sensitivity among different species (e.g., Posthuma et al. 2002) as well as very complex applications of environmental fate models (e.g., Randall et al. 2003). Also, although elementary Monte Carlo Analyses can be conducted in commonly available software programs like EXCEL, most Monte Carlo analyses require relatively specialized software. The above example was conducted using an EXCEL add-in called Crystal Ball (Decisioneering 2004) that is commonly used in probabilistic risk assessments conducted by or for the U.S. EPA's Office of Pesticides, Environmental Fate and Effects Division. Other packages capable of more sophisticated modeling include acslXtreme (AEgis Technologies Group 2004), ModelMaker (Cherwell Scientific 2000), and Mathematica (Wolfram Research 2004).

1.2.2.2. Extreme Value Risk Assessment – The USDA Forest Service has not adopted probabilistic risk assessment methods. Historically, the Forest Service has developed different scenarios that have been referred to as typical and worst-case (e.g., USDA/FS 1989a,b,c). With the advent of the SERA risk assessments, a somewhat different approach was taken in which almost no values used in a risk estimate are presented as a single number. Instead, most numbers used in calculating risk values are expressed as a central estimate and a range, which is sometimes very large. The central estimate would generally correspond to the *typical* value and the upper value in the range (or more specifically the upper or lower bound that leads to the highest estimate of risk) would generally correspond to what used to be called the “*worst-case*” value. The other end of the range (the upper or lower bound that leads to the lowest estimate of risk) might be termed the “*best case*” value. The best case assessment is made simply because an unacceptable level of risk from a *best case* would lead to the clear conclusion that the use of the agent under any circumstances would likely result in some adverse effect.

As with a probabilistic risk assessment, an attempt is often made to apply the extreme value approach both to the exposure assessment as well as to the dose-response assessment. Applications of the exposure assessment are relatively simple and may involve various assumptions concerning animal weight, food consumption, water consumption, rainfall and so forth. Many of the specific assumptions are detailed in Section 3.2 (Human Health) and Section 4.2 (Ecological Effects). In terms of the dose-response assessment, the extreme value approach

most often involves the identification of both tolerant and sensitive species, typically in the ecological risk assessment (Section 4.3). In the human health risk assessment (Section 3.3), different RfD values may be derived for sensitive subgroups – e.g., children or women of child-bearing age.

The extreme value approach has some but not all of the benefits of probabilistic risk assessment. For example, it can and often does indicate that a particular use of an agent might not cause any adverse effects under some circumstances but could cause adverse effects under other circumstances. To the extent that the circumstances are clearly defined, this may serve as a guide to using the agent in a manner that will minimize the potential for adverse effects. While probabilistic risk assessments may be used by the Forest Service at some point in the future, probabilistic risk assessments generally take longer to conduct (because of the tiered nature of the risk assessment process) and involve the commitment of greater resources.

1.3. LITERATURE SEARCH

1.3.1. Open Literature

There are many commercial databases that can be used to search the published literature. Initially, SERA conducts on-line searches of TOXLINE (including PubMed) and AGRICOLA. These two data bases usually identify most of the relevant published literature. Other supplemental searches may be conducted using other commercial data bases as detailed below.

TOXLINE (Toxicology Literature Online) is a bibliographical database constructed by the U.S. National Library of Medicine (NLM). The database is available at: <http://toxnet.nlm.nih.gov/>. The database covers the pharmacological, biochemical, physiological, and toxicological effects of agricultural and industrial chemicals, drugs, and several other classes of specialty chemicals. TOXLINE is a collection of databases derived from BIOSIS (up to 2002), National Library of Medicine, American Society of Hospital Pharmacists, National Institute for Occupational Safety and Health, Environmental Mutagen Information Center, Environmental Teratology Information Center, and U.S. Environmental Protection Agency. Sources for the BIOSIS sub-file include journal articles, reviews, reports, and meeting papers. The sources of information in TOXLINE include journal articles, letters, meeting abstracts, monographs, research and project summaries, technical reports, theses, and unpublished materials.

The specific component databases included in TOXLINE are listed below with the most relevant files in bold type:

ANEUPL	Aneuploidy File, Environmental Mutagen Information Center, Oak Ridge National Laboratory, 1970-1986
BIOSIS	Toxicological Aspects of Environmental Health, BIOSIS, from 1970 to the present.
CIS	CIS Abstracts, International Labour Office, International Occupational Safety and Health Information Center, 1981 to the present

CRISP	Toxicology Research Projects, National Institutes of Health, FY89-91
DART	Development and Reproductive Toxicology File, from 1989 to the present
EMIC	Environmental Mutagen Information Center File, Oak Ridge National Laboratory, from 1950 to the present
EPIDEM	Epidemiology Information System, FDA Center for Food Safety, 1940 to the present
ETIC	Environmental Teratology Information Center File, Oak Ridge National Laboratory, from 1950 to the present
FEDRIP	Federal Research in Progress, National Technical Information Service (NTIS), from 1990 to present
HMTC	Hazardous Materials Technical Center File, Defense Logistics Agency, 1982 to the present
IPA	International Pharmaceutical Abstracts, American Society of Hospital Pharmacists, from 1969 to the present
NTIS	Toxicology Document and Data Depository File, National Technical Information Service, from 1979 to the present
PESTAB	Pesticides Abstracts (formerly Health Aspects of Pesticides Abstract Bulletin), Environmental Protection Agency, 1968-1981
PPBIB	Poisonous Plants Bibliography, a special collection of mostly pre-1976 material on this subject prepared especially to complement more recent coverage by other subfiles
RISKLINE	National Chemicals Inspectorate (KEMI) in Sweden
TOXBIB	Toxicity Bibliography, National Library of Medicine, from 1966 to the present
TSCATS	Toxic Substances Control Act Test Submissions, pre-1988 to the present

Although TOXLINE is particularly useful for identifying much of the mammalian toxicology and general information on chemical and physical properties, it offers less comprehensive coverage of information on ecotoxicology and environmental fate. This information, however, is provided in AGRICOLA, a bibliographical database of citations covering the agricultural and forestry literature. AGRICOLA, which was created and is maintained by the National Agricultural Library, is the most comprehensive database of bibliographical information available in agricultural research. The multi-disciplinary coverage of AGRICOLA, which reflects the contents of the National Agricultural Library and other agricultural and scientific institutions, comprises more than 1400 international journals. The database consists of publications and resources in agriculture, animal and veterinary sciences, entomology, plant sciences, forestry,

aquaculture and fisheries, farming and farming systems, agricultural economics, extension and education, food and human nutrition, and earth and environmental sciences.

AGRICOLA is searchable at no cost at <http://agricola.nal.usda.gov/>. This source, however, does not offer the download flexibility of AGRICOLA offered by a commercial firm, Community of Science (COS). SERA maintains a commercial account with COS and uses this account for all literature searches of AGRICOLA.

For some very specialized searches, SERA may occasionally use other databases, most notably DIALOG and CAS ONLINE. Dialog Information Services, Inc. is one of the primary information retrieval systems on the commercial market. More than 250 databases are available for direct on-line retrieval (e.g., SciSearch, CA Search), containing more than 45,000,000 records. Records, or units of information, can range from directory-type information on chemical manufacturing plants to a citation with complete bibliographical information, and in many cases an abstract referencing a specific journal, conference paper, or other original source.

CAS ONLINE, available through STN International, is the only search system to function as a true on-line equivalent of the printed *Chemical Abstracts*. Almost anything that can be searched in the printed *Chemical Abstracts* dating back to 1967 can be searched on the CAS ONLINE CA File. CAS ONLINE permits access to the CAS Chemical Registry System by structure diagram, chemical name, CAS Registry Number and search terms for concepts, processes and other subject-related terms. CAS Registry, a world class database of chemical substances, contains more than 27 million records, including more than 16 million organic and inorganic substances. Chemical Abstracts (CA) is the largest and most current database of chemical information with approximately 16 million abstracts of journal articles, patents, and other chemical information documents. CA sources include more than 8000 worldwide journals, patents, technical reports, books, conference proceedings, and dissertations.

1.3.2. FIFRA/CBI Studies

For many pesticides, particularly those developed only in the past decade, the most relevant and critical information is found in unpublished studies submitted by the registrant of the pesticide to the U.S. EPA as part of the registration package. These studies are classified as “Confidential Business Information” and cannot be accessed without special clearance from the U.S. EPA. Summaries of these studies in the form of “one-liners” or Data Evaluation Records (DERs) usually are available through a Freedom of Information Act (FOIA) request. Sometimes, summaries of certain CBI studies are published by the U.S. EPA in Federal Register notices, Reregistration Eligibility Decision (RED) documents, or other Agency publications such as Science Chapters prepared by Health Effects Division or the Environmental Fate and Effects Division of the Office of Pesticides.

Although SERA sometimes obtains DERs and uses U.S. EPA summaries to reflect the views of the U.S. EPA, SERA does not rely on these summaries for an evaluation of the studies. SERA usually requests (by direct acquisition or FOIA) about 50-75% of the registration package and

personally reviews the studies. Within the limits of the FIFRA statute, SERA summarizes as much of this information as possible in appendices that accompany all full risk assessments. In addition to reviewing the CBI studies, SERA discusses the available information on the chemical with members of U.S. EPA/OPP in order to clarify technical issues in the data evaluation.

A major advantage of the FIFRA studies submitted for pesticide registration is that they follow a relatively uniform set of guidelines or study protocols. Some of the specific components of these guidelines have evolved over time and continue to be modified as needed. A summary of recent guidelines is given by U.S. Environmental Protection Agency's Office of Prevention, Pesticides, and Toxic Substances (U.S. EPA/OPPTS 2005) and are available at: www.epa.gov/OPPTS_Harmonized/. These guidelines are intended to constitute a consistent set of study standards that are used by both Office of Pesticide Programs (OPP) as well as Office of Pollution Prevention and Toxics (OPPT). A very large number of guidelines are available in ten different areas:

The OPPTS harmonized guidelines are organized in the following 10 series:

- 810 - Product Performance Test Guidelines
- 830 - Product Properties Test Guidelines
- 835 - Fate, Transport and Transformation Test Guidelines
- 840 - Spray Drift Test Guidelines
- 850 - Ecological Effects Test Guidelines
- 860 - Residue Chemistry Test Guidelines
- 870 - Health Effects Test Guidelines
- 875 - Occupational and Residential Exposure Test Guidelines
- 880 - Biochemicals Test Guidelines
- 885 - Microbial Pesticide Test Guidelines

Forest Service risk assessments primarily involve Health Effects Test Guidelines (Series 870), Ecological Effects Test Guidelines (Series 850) and Fate, Transport and Transformation Test Guidelines (Series 835). While it is beyond the scope of the current document to discuss all of these guidelines in detail, specific guidelines are discussed as necessary and referenced to U.S. EPA/OPPTS (2005). For example, the guideline for acute oral toxicity tests (discussed in Section 3.1.4 and Section 4.1.2.1) is entitled *Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity* with the EPA report designation of EPA 712-C-96-190. In the current document, this is referred to simply as OPPTS 870.1100.

1.3.3. Credible Reviews

For the most part, the risk assessments are based on primary literature, either from the open or published literature (Section 1.3.1.1) or the FIFRA files (Section 1.3.1.2). In some cases, however, credible reviews may be used directly as both a source of information and as the basis for the risk assessment. This may be done for some impurities or adjuvants for which there is a very large body of literature or for abbreviated risk assessments. Conducting a full risk

assessment on some of these agents may not be feasible based on resource limitations. In addition, some of these agents may be the subject of extensive reviews and risk assessments by other agencies or organizations and it simply would not make sense to duplicate the effort. In general, credible reviews are limited to groups such as the U.S. EPA, the World Health Organization (WHO), and the Agency for Toxic Substances and Disease Registry (ATSDR).

The U.S. EPA has conducted a very large number of reviews on pesticides (e.g., <http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg>) and industrial chemicals that may be used in pesticide formulations (e.g., <http://www.epa.gov/iriswebp/iris/index.html>). Because of the unique role and legislative mandate of the U.S. EPA in the conduct of risk assessments that are typically subject to extensive review and deliberation, the Forest Service will often defer to the U.S. EPA on evaluation and selection of studies used in the dose-response assessment for both human health (Section 3.3) and ecological effects (Section 4.3). This allows Forest Service risk assessments to focus the analysis of uses of the agent that are specific to the program activities of the Forest Service.

ATSDR is part of the Centers for Disease Control (CDC) and was created by Congress to provide public health-related analyses specifically related to hazardous wastes and environmental spills of hazardous substances. Part of these activities include the preparation of toxicological profiles for hazardous substances. While many of the compounds reviewed by ATSDR are industrial compounds rather than pesticides, some compounds reviewed by ATSDR are pesticide contaminants. As an example, ATSDR has an extensive review of hexachlorobenzene, which is a contaminant in two herbicides, picloram and clopyralid. In both of these Forest Service risk assessments, the ATSDR review of hexachlorobenzene was used extensively.

The World Health Organization has conducted a very large number of reviews on both pesticides and industrial chemicals under the Programme on Chemical Safety (IPCS). Environmental Health Monographs on a large number of compounds are available at: <http://www.inchem.org/pages/ehc.html>. The preparation of these monographs typically involves several primary authors from academia or government and the process is reviewed by a larger group of individuals with expertise on the particular agent. A major benefit of WHO reviews is that they often contain summaries of unpublished studies from Europe that were submitted to the WHO in support of the monograph preparation. Unlike documents prepared by U.S. EPA and ATSDR, the Forest Service risk assessments will typically use WHO reviews only as an information source and does not typically use risk assessment values derived in WHO documents directly in the risk assessment.

1.3.4. Other Secondary Sources and Gray Literature

SERA searches various Internet sites in addition to the published and unpublished literature. The Internet is a major source of information for many other U.S. government agencies (e.g., USGS). In addition to government sites, the web sites of some chemical manufacturers and environmental groups contain information pertinent to the risk assessment. SERA uses discretion in identifying reliable sources of information and clearly identifies those sources in the risk assessment.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

Program descriptions are relatively brief discussions about the pesticide under review and how the Forest Service plans to use the pesticide. The information summarized in the program description includes the identity of the pesticide and its commercial formulations, as well as the identity of the inerts, adjuvants, and contaminants in the commercial formulations. SERA contacts one or more individuals in the Forest Service to obtain information about how the pesticide will be used in Forest Service Programs. Typically, a draft of the program description is prepared and reviewed by Forest Service personnel prior to the preparation of the rest of the risk assessment to ensure that the risk assessment is based on Forest Service practices. The program description may include additional information about the use of the pesticide by other organizations, which can be useful in assessing the extent to which the application of the pesticide by the Forest Service contributes to the environmental levels of the compound.

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

In the program description, the identity of the pesticide (i.e., active ingredient) is summarized followed by a brief discussion of the commercial formulations. The discussion of the commercial formulations includes information about the proportion or concentration of the active ingredient in each formulation as well as a general description of the formulation(s) (e.g., physical state—liquid, dispersible granules, etc.—and type of carrier or binding matrix).

Physical and chemical properties that are environmentally significant and probably of greatest relevance to most risk assessments include the vapor pressure, ionization constants (pK_a), water and lipid solubility, and adsorption properties (e.g., K_d , K_{oc} , and K_{ow}). SERA obtains most of the information regarding the physical and chemical characteristics of a compound from the U.S. EPA/CBI files and standard reference sources like the Merck Index (Budavari 1989) or the USDA ARS Pesticide Database (<http://ncsr.arsusda.gov/ppdb3/>). In some instances, quantitative structure-activity relationships, such as the U.S. EPA's EPI-Suite program (Clements et al. 1996; Meylan and Howard 1998, 2000;) may be used to estimate physical and chemical properties for which experimental data are not directly available. Data regarding chemical reactivity (e.g., rates of hydrolysis, biodegradation, photodegradation, etc.) and monitored rates of environmental dissipation of the pesticide also are included in this section of the program description. In addition, SERA conducts supplemental literature searches (e.g., the CHEMLINE database available online via the National Library of Medicine) as necessary to obtain information about chemical structures and nomenclature.

The chemical and physical properties of a pesticide are summarized in a table that also includes the name of the compound, synonyms, the CAS number(s), and U.S. EPA registration number. If necessary, the table also indicates the conditions under which certain measurements were made. For example, the solubility of weak acids in water is highly dependent on the pH of the water. Similarly, soil-water partition coefficients vary substantially for different soil types such as clay, loam, and sand. Generally, the program description does not include a detailed discussion of the chemical or physical properties of an agent. When necessary, those kinds of

discussions may be incorporated into the exposure assessment. If GLEAMS modeling is conducted, an additional table defining the chemical and physical properties used in the model is included in the document.

The program description also addresses the issue of inerts in commercial pesticide formulations, which are regulated by the U.S. EPA (Levine 1996). The regulations affect pesticide labeling and testing requirements. As part of its regulatory activity, the U.S. EPA classifies inerts into one of four lists, based on available toxicity data (www.epa.gov/opprd001/inerts/lists.html). Although the lists are useful for setting testing requirements and, perhaps, in encouraging the use of inerts with low inherent toxicity, they do not explicitly consider the potential effects of the inerts on the toxicity of the formulation.

Most chemical manufacturers consider the identity of inert ingredients proprietary information. Inert compounds classified as hazardous by the U.S. EPA must be specified on the MSDS when they are present at a concentration greater than 0.1%. A lack of disclosure means that none of the inert ingredients present at concentrations greater than 0.1% in the formulation are classified as hazardous. As discussed by Levine (1996), the testing requirements for inerts are less rigorous than the testing requirements for active ingredients.

The identity of the inerts is always disclosed to the U.S. EPA as part of the registration process. Although SERA obtains and reviews this information while preparing the risk assessment, SERA does not disclose specific information about the inerts in the risk assessment.

Information about the impurities in technical grade pesticides also must be submitted to the U.S. EPA. SERA obtains and reviews this information while preparing the risk assessment. Since the identities of the impurities also are considered proprietary, SERA does not disclose this information in the risk assessment document; however, the potential impact of impurities on the risk assessment is discussed in the hazard identification section of the document (Section 3.1.15).

2.3. APPLICATION METHODS

The use of herbicides in silviculture and the various methods of herbicide application are described in detail in the general literature (e.g., Cantrell and Hyland 1985), and in environmental impact statements conducted by the Forest Service (e.g., USDA 1989a,b,c). No attempt is made to summarize this information again in the risk assessment. Instead, SERA discusses information relevant to the exposure assessments (section 4) for application methods that the Forest Service uses or may consider using.

Generally, consideration is given to three conventional application methods, including directed foliar applications, broadcast ground applications, and aerial applications. The rationale for selecting these basic application methods is discussed in SERA (1998). Sometimes, as with the application of granules (e.g., hexazinone) or the application of a compound directly to water (e.g., 2,4-D), additional application methods are described.

For each application method, this section of the risk assessment focuses on the number of acres that an individual worker might handle in a single work day and any special precautions that may be employed routinely. SERA obtains this information from descriptions of pesticide applications provided by the Forest Service (e.g., USDA 1989b, p 2-9 to 2-10) and any chemical-specific or site-specific information provided by the Forest Service.

For example, in selective foliar applications, the herbicide sprayer or container is carried by backpack and the herbicide is applied to selected target vegetation. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposure, application crews are directed not to walk through treated vegetation. Usually, a worker treats approximately 0.5 acre/hour with a plausible range of 0.25-1.0 acre/hour.

Boom spray or broadcast ground applications are used primarily in rights-of-way management. Spray equipment mounted on tractors or trucks is used to apply the herbicide on either side of the roadway. Usually, about 8 acres are treated in a 45-minute period (approximately 11 acres/hour). Some special truck mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of herbicide mixture (approximately 21 acres/hour and 510 gallons/hour)

Aerial applications are made with helicopters or fixed wing aircraft. The compound is applied under pressure through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. In aerial applications, approximately 40-100 acres may be treated per hour.

2.4. MIXING AND APPLICATION RATES

In this section of the program description, SERA briefly discusses information provided by the Forest Service regarding proposed application rates and pesticide concentrations in field solutions.

The specific application rates used in ground or aerial applications vary according to local conditions and the nature of the target vegetation. SERA does not derive the application rates, but refers to the product labels to ensure that the proposed application rates do not exceed the labeled rate for a particular use. Moreover, SERA checks all supplemental labels to ensure that special restrictions on use within different geographical areas are clearly stated in the risk assessment. This kind of information is obtained either directly from literature released by the manufacturer or from C&P Press at <http://www.greenbook.net>.

In most risk assessments, only a single application rate is explicitly considered in the exposure assessments (Section 3.2 and 4.2). This is based either on information obtained from the Forest Service on planned used or past records of Forest Service use (Section 2.4). The consequences of varying application rates within the range of rates that the Forest Service may use are

considered in the risk characterization for human health (Section 3.4) and ecological effects (Section 4.4).

Usually, pesticides are diluted prior to field applications. This detail is referred to in the risk assessment as *field dilution*. For example, the recommended range of mixing volumes for many liquid pesticide formulations is about 5-25 gallons of water per acre for aerial applications and about 10-100 gallons of water per acre for ground applications.

For the risk assessment, the extent to which a formulation is diluted prior to application primarily influences dermal and direct spray scenarios, both of which depend on the field dilution. The greater the concentration of pesticide in the applied solution, the greater the exposure and the greater the risk. Like application rates, field dilutions are generally expressed as a range with a central or average value.

It should be noted that the selection of a specific application rate and dilution volume in a risk assessment is intended to simply reflect typical or central estimates as well as plausible lower and upper bounds. In the assessment of specific program activities, the Forest Service may use program specific application rates in the worksheets that are included with each risk assessment to assess any potential risks for a proposed application. Details of these worksheets are given in Appendix 3.

2.5. USE STATISTICS

The program description provides two kinds of statistical data regarding pesticide use: past use by the Forest Service for the most recent year where data are available, including information on tank mixtures, when obtainable; and total national or regional use of the pesticide. The Forest Service provides statistics on the annual use of pesticides. Data regarding total and regional pesticide use data are available from various sources. Agricultural use data is generally available at the U.S. Geological Service web site: www.dwatcm.wr.usgs.gov/cppt/.

Although neither the statistics pertaining to pesticide use by the Forest Service nor the statistics pertaining to total national or regional pesticide use have a direct impact on the risk assessment, they can be useful in interpreting and better understanding the results of the risk assessment. For example, in a recent Forest Service risk assessment on clopyralid, SERA assessed the potential significance of hexachlorobenzene, a contaminant in clopyralid. By assessing the amount of clopyralid that the Forest Service is likely to use and the total amount of hexachlorobenzene released to the environment each year from all sources, SERA demonstrated that the Forest Service programs would contribute about one part in one-hundred million (100,000,000) parts of the total hexachlorobenzene release.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

The hazard identification process involves making judgments about which effects are most relevant to the assessment of human health. During this process, studies may be eliminated from consideration because they are inherently flawed or because they are grossly inconsistent with the preponderance of other studies. Although hazard identification results in a qualitative determination, quantitative methods are usually required as in most other assessments of causality. For instance, the process of hazard identification often hinges on a statistical assessment of exposure-response or dose-response relationships. Furthermore, hazard identification must also consider fundamental and qualitative differences among species. Depending on the chemical of concern, hazard identification also may include the use of quantitative or qualitative structure-activity relationships or differences in pharmacokinetics.

The hazard identification may cover any number of endpoints, depending on the chemical under assessment. The following topics are generally considered explicitly in each hazard identification:

- Mechanism of Action
- Pharmacokinetics and Metabolism
- Acute Oral Toxicity
- Subchronic or Chronic Systemic Toxic Effects
- Effects on the Nervous System
- Effects on the Immune System
- Effects on the Endocrine System
- Reproductive and Teratogenic Effects
- Carcinogenicity and Mutagenicity
- Irritation and Sensitization of the Skin and Eyes
- Systemic Toxic Effects from Dermal Exposures
- Systemic Toxic Effects from Inhalation Exposures
- Inerts and Adjuvants
- Impurities and Metabolites
- Toxicologic Interactions

Additional effects may be discussed, depending on the nature of the available information on the chemical. Most standard texts in toxicology provide overviews of the diverse nature of the effects on different organs (e.g., Klaassen 1996, Haschek and Rousseaux 1991).

3.1.2. Mechanism of Action

Mechanism of action is a rather general term that refers to our understanding of how a particular chemical is likely to affect humans or other organisms. To the extent that the mechanism of action is understood, confidence in a risk assessment is enhanced. If the mechanism is not

understood, extrapolations or suppositions concerning levels of exposure that may or may not cause an adverse effect are less certain.

The mechanism of action can be described at many levels of biological organization: molecular, biochemical, sub-cellular (e.g., effects on organelles such as mitochondria), cellular, organ, organ system or whole animal. This section of the risk assessment attempts to summarize this information in a manner that focuses attention on the following sections of hazard identification. Thus, even if the mechanism of action is not clearly defined at the molecular level, some attempt is made to characterize the general types of effects that are most often seen and to suggest whether or not these observations might be related to a plausible mechanistic assumption.

For many pesticides considered in Forest Service risk assessments, the mechanisms of action may be very well understood in the target species (e.g., the effect of an herbicide on a plant or the effect of an insecticide on an insect) but these mechanisms may have very little to do with potential human health effects. For example, many sulfonylurea herbicides inhibit acetolactate synthase (ALS), an enzyme that catalyzes the biosynthesis of three branched-chain amino acids (valine, leucine, and isoleucine), all of which are essential for plant growth. In terms of potential effects in humans, all of these amino acids are essential amino acids – i.e., amino acids that are not produced by humans and must be obtained from the consumption of plants. Thus, the mechanism of action of the sulfonylurea herbicides in plants has no direct relevance to the human health risk assessment.

3.1.3. Pharmacokinetics and Metabolism

Pharmacokinetics refers to the study of how chemicals may be absorbed, distributed, altered (metabolized), and excreted. From a practical perspective, this section of the risk assessment focuses on what is known about metabolism, absorption (particularly dermal absorption), and excretion.

3.1.3.1. Metabolism – Consideration of metabolism focuses on similarities between metabolism in humans and metabolism in experimental animals. This is a very important issue to most risk assessments, because toxicity studies in experimental animals are typically used to derive acceptable levels of exposure in humans (Section 3.3). Implicit in this practice is the assumption that studies on whole animals, such as those used to derive acceptable levels of exposure in humans, will encompass the toxicity of both the parent compound as well as any metabolites that formed *in vivo*. For chemicals that are extensively metabolized and chemicals whose metabolites are known to be more toxic than the parent compound, this assumption can be supported by information showing that the metabolic pathways in humans and experimental mammals are similar. To the extent that the metabolic pathways in humans and experimental mammals differ, confidence in use of data on experimental mammals and subsequent confidence in the risk assessment itself may be diminished.

In any risk assessment, a major distinction is made between *in vivo* and environmental metabolites. *In vivo* metabolites, as discussed in this section, refer to the compounds that are

formed within the animal after the agent has been absorbed. Environmental metabolites refer to compounds that may be formed in the environment by a number of different biological or chemical processes including breakdown in soil or water or breakdown by sunlight (photolysis). In many cases, environmental metabolites will be less biologically active than the parent compound. Thus, environmental metabolism is regarded as a detoxification mechanism and the metabolites are not quantitatively considered in the risk assessment. In other cases, such as the formation of 3,5,6-trichloro-2-pyridinol (TCP) from triclopyr, the metabolites may be as toxic or more toxic than the parent compound. In such cases, the metabolite or metabolites may need to be treated in the same manner as the parent compound – i.e., a full exposure assessment and dose-response assessment is required.

A major uncertainty in this type of assessment on metabolites involves the kind of toxicity data available on metabolites. The example of TCP as a metabolite of triclopyr is exceptional in that information is available on both the acute and chronic toxicity of TCP. For most pesticide metabolites either no toxicity data are available or the toxicity data are limited to acute toxicity data (Section 3.1.4). As discussed in Section 1.2.1, a fundamental principle in the Forest Service risk assessments is that risk cannot be quantitatively characterized unless both toxicity and exposure can be characterized. Thus, in cases in which inadequate toxicity data are available to quantify the risks associated with one or more metabolites, the metabolites are not quantitatively considered in the risk assessment and any attendant uncertainties are discussed in the risk characterization (Section 3.4).

3.1.3.2. Absorption – In most cases, chemicals may be absorbed by oral, dermal, and inhalation routes. From a practical perspective, only dermal exposures are quantitatively considered in this section of the risk assessment. This approach is taken because route-to-route extrapolations are made in the risk assessments only for dermal exposures. In other words, oral exposures are estimated in units such as mg/kg/day (Section 3.2) and these exposures are compared to toxicity values based on oral exposures (Sections 3.1.4 and 3.1.5). While inhalation exposures are not typically important for most pesticides (e.g., Ecobichon 1998; van Hemmen 1992), any relevant inhalation exposures are typically compared to inhalation toxicity studies (Section 3.1.13).

In Forest Service risk assessments, however, the potential effects of dermal exposures are typically assessed using oral toxicity data. This is one area in which Forest Service risk assessments tend to differ from those conducted by the U.S. EPA's Office of Pesticides (OPP). Most often, the U.S. EPA/OPP will use dermal toxicity data to assess the potential for adverse effects in humans. Thus, as with the oral and inhalation exposures discussed above, no route-to-route extrapolation is required. Forest Service risk assessments, on the other hand, will take a dermal exposure and estimate an absorbed dose. This absorbed dose is then compared to oral toxicity values in making the risk characterization. The risk assessments conducted by the Forest Service take this approach because the dermal toxicity data on most chemicals, including herbicides and other pesticides, is much more limited than the data from oral toxicity studies. For example, reproductive effects are of critical concern to the Forest Service but very few reproductive studies (Section 3.1.9) are conducted using dermal exposures. While dermal

toxicity data is considered in Forest Service risk assessments (Section 3.1.12), they are most often used as a check on estimates of dermal absorption rates (see below) rather than as the basis for a risk characterization.

In making dermal-to-oral extrapolations, estimates of dermal absorption rates are critical. In the Forest Service risk assessments, estimates of dermal absorption rates (k_a , expressed in units of amount/unit time [zero-order] or reciprocal time [first-order]) or dermal penetration rates (K_p , expressed in units of cm/hour) are required for many of the exposure scenarios. The biological and chemical processes pertinent to these scenarios are illustrated in Figure 3-1. The chemical may be deposited on the skin instantaneously (e.g., as in an accidental spill) or gradually (e.g., as uptake from contaminated vegetation). In order for absorption in the systemic circulation to occur, *permeation* across the stratum corneum must occur first—at least in intact skin.

The stratum corneum and dermis are basically lipid-rich barriers that prevent water loss. Thus, compounds with a high lipid solubility are generally more permeable than more water soluble compounds. In addition, transport through the skin is inversely related to molecular size. Thus, for compounds of comparable lipophilicity, smaller compounds tend to be more permeable than larger compounds.

Classical pharmacokinetic dermal absorption rates are used to estimate the absorbed dose associated with dermal deposition scenarios. These rates (k_a) express the amount (zero-order absorption) or proportion (first-order absorption) of a chemical absorbed *into the body* per unit time. In this context, *into the body* means that the chemical will be in the blood stream and subject to metabolism or excretion and capable of interacting in other ways with viable tissue.

As discussed in U.S. EPA (1992), most QSAR relationships for estimating dermal permeability (K_p) take these relationships into account with dermal permeability being positively related to the octanol/water partition coefficient (K_{ow}) and inversely related to molecular weight (MW). U.S. EPA (1992) recommends the following equation:

$$\log_{10} K_p = -2.72 + 0.71 \log_{10} K_{ow} - 0.0061 \text{ MW} \quad (\text{Eq. 3-1})$$

where K_p is in units of cm/hour. This equation is based on measured K_p values for 95 organic compounds (Flynn 1990, Table 5-4 in U.S. EPA 1992) with $\log K_{ow}$ values ranging from about -2.5 to 5.5 and molecular weights ranging from about 30 to 770. Estimates of K_p from the above equation have an error of about one order of magnitude.

As reviewed by U.S. EPA (1992), some analyses (e.g., Flynn 1990) suggest that the effects of both molecular weight and lipophilicity on permeability may be linear only within certain limits. Based on the analysis by Flynn (1990), relatively lipophobic compounds with $\log K_{ow}$ values <0.5 appear to have $\log K_p$ values of approximately -3 (MW<150) or -5 (MW>150). At the upper limit, highly lipophilic compounds with $\log K_{ow}$ values >3 and molecular weights <150

appear to have log K_p values of about -0.5. Compounds with log $K_{o/w}$ values >3.5 and molecular weights >150 appear to have log K_p values of about -1.5 (Flynn 1990).

The series of studies by Feldmann and Maibach (1969, 1970, 1974) represents a unique and highly relevant source of information on *in vivo* dermal absorption in humans. As discussed in U.S. EPA (1992), however, the Feldmann and Maibach publications do not provide sufficient experimental details for the complete derivation of zero-order dermal absorption rates. Nonetheless, as illustrated by Durkin et al. (1995), estimates of dermal absorption rates from the Feldmann and Maibach publications gave much better estimates of absorbed dose than did estimates based on Fick's first law. Thus, when exposure scenarios are best characterized by deposition on the surface of the skin – as opposed to immersion of the skin in an aqueous solution – first order dermal absorption rates are estimated either from chemical specific data or from structure activity relationships.

SERA (1997) has completed an extensive re-evaluation of these data to improve on the methods proposed by Durkin et al. (1995) in which the Feldmann and Maibach data were fit to the following equation:

$$X_t = [k_a A_0 / (k_e - (k_a + k_r))] (e^{-(k_a + k_r)t} - e^{-k_e t}) \quad (\text{Eq. 3-2})$$

where k_a is the first order dermal absorption rate coefficient, k_e is the first order excretion rate coefficient, and k_r is the first order fugitive loss rate coefficient

Feldmann and Maibach (1969, 1970, 1974) did not conduct i.v. elimination studies in humans for all of the compounds. For some of the compounds i.v. studies were conducted in rats and for other compounds judgement was used to estimate k_e . Thus, in the re-analysis, only the 29 chemicals that included i.v. elimination studies in humans are included in the analysis.

For each of these 29 chemicals, a spreadsheet was set up in Excel and the Excel SOLVER function was used to estimate the rate coefficients. Because the results reported in the Feldmann and Maibach publications are expressed as the proportion of applied dose eliminated over a given period, both sides of the above equation were multiplied by k_e . In all cases, the k_e values were derived from the half-times ($t_{1/2}$) reported in the Feldmann and Maibach publications - i.e., $k_e = \ln(2) \div t_{1/2}$ - and these k_e values were used as constants rather than as parameters estimated from the models. The only constraint applied to the models was that k_a and k_r both must be greater than or equal to zero.

Unlike the earlier results of Durkin et al. (1995), first-order absorption rate coefficients were best estimated based on both molecular weight and log $K_{o/w}$:

$$\log_{10} k_a = -1.49 + 0.233 \log_{10} K_{o/w} - 0.00566 \text{ MW} \quad (\text{Eq. 3-3})$$

All coefficients were significant at $p < 0.004$, but the squared correlation coefficients for both models were low, about 0.32. This correlation coefficient is not remarkably lower than the squared correlation coefficient of 0.43 that is obtained for the regression of $\log K_p$ on molecular weight and $\log K_{o/w}$ using Table 5-7 from U.S. EPA (1992) without censoring. The fugitive loss rates (k_r) were not significantly correlated with either the molecular weight or the $K_{o/w}$. The observed fugitive loss rates fit a log normal distribution [$p=0.35$] with a mean of 0.032 hour^{-1} and a 95% confidence interval of 0.0028 to 0.037 hour^{-1} .

Although there is no information with which to compare the absorption of the esters of weak acids with the acids themselves, Feldmann and Maibach (1969) did assay the absorption of hydrocortisone and testosterone as well as esters of these compounds (Table 3-1).

As indicated in Table 3-1, hydrocortisone and hydrocortisone acetate show a relatively direct relationship between dermal permeability (K_p) and dermal absorption. For testosterone and its esters, however, the correspondence is poor. Although the estimated K_p for testosterone is less than that for either of its esters, testosterone is absorbed to a substantially greater extent than either of its esters. This relationship holds true whether the estimates of K_p for the esters are based on Equation 5 or the upper limit on K_p suggested by Flynn (1990). Thus, while the lipophilicity of the esters is greater than that of the parent compound for both testosterone and hydrocortisone, and the esters of both of these compounds are estimated to have a greater permeability (K_p) than the corresponding parent compound, the relationship of ester formation to dermal absorption is inconsistent.

Many factors can influence the dermal penetration and dermal absorption of chemicals, and some of these factors may be useful in understanding the lack of a consistent relationship between dermal permeability and dermal absorption. U.S. EPA (1992) provides an overview of these factors, and additional information and analyses are presented in other reviews and books on dermal absorption (e.g., Klein-Szanto et al. 1991, Rice and Cohen 1996, Scott et al. 1989, Wang et al. 1993).

As illustrated in Figure 3-1, dermal absorption involves both permeation of the epidermis as well as partitioning from the dermis into capillary blood. At least to some extent, this process will be affected by the relative differences in the fat and water content of the skin and blood. As illustrated in Figure 3-2, whole skin tissue contains about 10% fat [260 g/2600 g] and 61% water [1600 g/2600 g] (ICRP 1992, Table 105, p. 284). The outer layer of the skin, the stratum corneum, contains almost 20% fat and 40% water (Klein-Szanto et al. 1991). Whole blood contains only about 0.65% fat [36 g/5500 g] and about 80% water [4400 g/5500 g] (ICRP 1992, Table 105, p. 280). Blood plasma contains about the same amount of fat as whole blood [23 g/3100 g or 0.74% fat] but a greater proportion of water [2900 g/3100 g or 93% water].

Because the skin, and especially the epidermis, is comprised of more lipids and less water than blood or plasma, increasing lipophilicity, which tends to increase dermal permeability or

penetration, will tend to decrease partitioning from skin into blood. Thus, it does not follow that apparent dermal absorption rates (k_a) will directly parallel dermal permeability (K_p).

The binding of the chemical to endogenous protein may also complicate the relationship between estimates of dermal permeability and dermal absorption. Skin is relatively rich in protein [750 g/2600 g or about 29%] (ICRP 1992, Table 105, p. 280). Plasma contains less but still significant levels of protein [210 g/3100 g or 6.7%], as does whole blood [990 g/5500 g or 18%]. Different chemicals may bind to different proteins with varying degrees of affinity. Moreover, skin and blood consist of different and multiple kinds of proteins. Compounds that bind tightly to some skin proteins may penetrate quickly into the dermis (high K_p) but partition rather slowly into blood plasma (low k_a). Conversely, if a chemical has a high affinity for plasma proteins, the concentration in the aqueous phase of the plasma will tend to diminish, favoring the partitioning from the dermis to the blood. Thus, the net effect of protein binding on dermal penetration or dermal absorption cannot be determined in the absence of specific data on the chemical of concern. Hence, protein binding is another factor that may account for apparent discrepancies between dermal absorption rates (k_a) and dermal permeability (K_p).

Another factor affecting the rate of dermal absorption involves penetration of the chemical through the epidermis to the dermis where absorption into the blood may occur. The epidermis is relatively thin, generally about 35 to 100 micrometers for men and 20 to 65 micrometers for women. In some parts of the body, like the fingers and soles of the feet, the epidermis is much thicker, ranging from 400 to 1400 micrometers for men and from 400 to 1000 micrometers for women (ICRP 1992, Table 6, p. 49). The consequences of different skin thicknesses are variability in permeation/absorption and a lag period in apparent absorption.

Furthermore, different parts of the body may have different rates of dermal absorption. Similarly, skin thickness and/or composition in the same part of the body may differ among individuals. The variability among individuals is likely to contribute to the observed inter-individual differences in dermal absorption rates. Differences in skin composition may also influence the permeability rate (K_p) of a compound either at different anatomic sites of an individual or at the same anatomic site among individuals (Klein-Szanto et al. 1991).

The other consequence of different skin thicknesses or differences in skin composition involves the apparent lag period between dermal exposure and dermal absorption. U.S. EPA (1992, p. 4-28) indicates that the apparent lag time for penetration of the stratum corneum is proportional to the square of the thickness of the stratum corneum and inversely proportional to the diffusiveness of a chemical within the stratum corneum. Although this relationship may adequately describe permeation (K_p), the rate of absorption is not likely to change in a quantal manner (i.e., remaining zero at times less than the 'lag time' and changing to a constant value at times greater than the lag time). In other words, penetration of the stratum corneum and functional saturation of the underlying skin tissue is not instantaneous. Initially, the functional absorption rate (k_a), which is assumed to be a constant under the assumption of zero- or first-order kinetics, may actually be

negligible but approach a constant value, either in terms of zero- or first-order coefficients, as permeation of the skin approaches a steady-state or psuedo-steady state.

The vehicle in which a compound is applied also may affect permeability and absorption. Moreover, these effects may be competing. There is ample evidence that some vehicles enhance dermal absorption and dermal permeability of various compounds, while other vehicles retard the processes (e.g., Walters 1989, Guy et al. 1989, Williams and Barry 1989). In general, vehicles that hydrate the skin or alter the physical state of the stratum corneum (e.g., some solvents) may enhance permeability. On the other hand, highly lipophilic vehicles may retard both permeability and the subsequent absorption of lipophilic compounds by impeding the partitioning of the chemical from the vehicle into the skin. The converse is true for highly lipophobic compounds in a lipophobic vehicle. Thus, the influence of a specific vehicle on *absorption* may not be related to its effect on *permeation*. These confounding factors may need to be addressed when data regarding the effects of various vehicles on dermal absorption are not consistent.

3.1.3.3. Excretion – The excretion of a chemical from an animal is also an important factor in assessing risk. Unlike dermal absorption rates, however, data on excretion rates are typically not used quantitatively in the risk assessment. Similar to information on metabolism, the assumption is generally made that the role of excretion is explicitly encompassed in the available toxicity data on an agent. In other words, data will generally be available on both acute toxicity (Section 3.1.4) as well as subchronic or chronic toxicity (Section 3.1.5). These data, along with information on effects of special concern (Sections 3.1.6 to 3.1.10) are then used to directly derive both acute and chronic toxicity values (Section 3.3).

Nevertheless, information on excretion rates, particularly whole-body excretion rates, can be useful in assessing the chemical-specific implications of the general terms *acute*, *subchronic*, and *chronic*. These terms do have general although somewhat vague definitions in toxicology. As discussed below (Sections 3.1.4 and 3.1.5), acute toxicity generally refers to single exposures or exposures that occur over a short period, typically 10-days or less. Subchronic toxicity is often defined somewhat circularly as 90-days because this is a period of exposure commonly used in studies that are referred to as *subchronic*. Other definitions of subchronic will sometimes define this exposure as about 10% of a life span, at least when referring to studies in rodents.

Pharmacokinetics can be used to define somewhat more biologically based characterizations of acute and chronic exposures in terms of body burden. Many compounds appear to be excreted from the body by first-order kinetics. In other words, a constant proportion of the compound is eliminated per unit time – e.g., 5% per day. The differential equation for this process is:

$$dA = -k_e A dt \quad (\text{Eq. 3-4a})$$

where A is the amount of the chemical, k_e is the first-order excretion rate constant, and dt is the period of time. In plain language, this equation simply states that the rate of change of the amount in the animal (dA) is equal to some constant $-k_e$ (in units of reciprocal time), times the

amount remaining in the animal (A), times the time interval, dt . At any particular time, T , the amount in the animal is simply the integral of Equation 3-3:

$$A_t = A_0 e^{-k_e T} \quad (\text{Eq. 3-4b})$$

where A_0 is the amount in the animal at time zero, immediately after dosing.

Intuitively, it should be apparent that the amount of a chemical in an animal after repeated doses will tend to increase more rapidly and to a greater extent as the elimination rate (k_e) decreases. This is the case, and the mathematics of this relationship has been formalized in the *plateau principle* (e.g., Goldstein et al. 1974). This principle can be applied to a compound that is eliminated by first-order kinetics (Eq. 3-3) when the compound is administered repeatedly at a fixed interval (t^*). In such a situation, the maximum amount of a chemical that will be in the animal after an infinite time (X_{inf}) can be calculated as:

$$X_{\text{inf}} = X_0 / (1 - e^{-k_e t^*}) \quad (\text{Eq. 3-5})$$

By simple algebraic rearrangement, the increase or concentration of the chemical in the body relative to the single dose can be calculated as:

$$X_{\text{inf}}/X_0 = 1 / (1 - e^{-k_e t^*}) \quad (\text{Eq. 3-6})$$

In applying this sort of relationship, it is useful to note that the halftime ($t_{1/2}$) can be defined as:

$$t_{1/2} = \ln(2)/k_e \quad (\text{Eq. 3-7a})$$

or

$$k_e = \ln(2)/t_{1/2} \quad (\text{Eq. 3-7b})$$

where \ln is the natural log (rather than the common log or \log_{10}) with $\ln(2)$ having a value of approximately 0.6931. Thus, a compound with a whole-body halftime of 2 days has a k_e of about 0.347 days^{-1} . Substituting this value into Equation 3-6 and assuming a dosing interval of one day ($t^*=1$), the maximum concentration of the chemical in the animal after an infinite number of doses would be 3.4. By comparison, a compound with a long halftime such as 1 year (365 days) would have a k_e of about 0.002 days^{-1} and substituting this value into Equation 3-6 leads to a concentration of about 527. Under the assumption that the critical factor for the animal is body burden, we would expect a substantial difference between the acute and chronic toxicity of a slowly eliminated chemical (e.g., a halftime of 1 year) but a lesser difference for a compound that is more rapidly eliminated (e.g., a halftime of 2 days).

The plateau principle can be extended to calculating the fraction (f) of the eventual steady-state condition that is reached after a certain period of time after n doses at a fixed interval:

$$f = 1 - e^{-k_e t^* n} \quad (\text{Eq. 3-8})$$

Thus, for a compound with a whole-body halftime of 2 days ($k_e = 0.347 \text{ days}^{-1}$), a 90 day exposure to doses administered each day ($t^* = 1 \text{ day}$) would result in a fraction of virtually 1. For a compound with a halftime of 1 year ($k_e = 0.002 \text{ days}^{-1}$), the fraction of the steady-state value would be only about 0.16. After a period of 2 years (730 days), the fractional value for the slowly eliminated compound would be about 0.76 or about 4.75 times higher than the 90-day value [$0.76/0.16 = 4.75$]. Again assuming that body burden is the critical factor, there would be a greater concern for the slowly eliminated compound compared to the rapidly eliminated compound that a subchronic study might underestimate the toxicity that could be seen in a full chronic study.

A rearrangement of Equation 3-8 may be used to express the time (or more properly the number of doses) to a given fraction of steady-state:

$$n = \ln(1-f)/-k_e t^* \quad (\text{Eq. 3-9})$$

Going back to the example of the rapidly eliminated compound ($k_e = 0.347 \text{ days}^{-1}$) and slowly eliminated compound ($k_e = 0.002 \text{ days}^{-1}$), this equation can be used to calculate the number of daily doses that would be required to reach 0.9 (90%) of the eventual steady-state value: about 6.6 days for the rapidly eliminated compound and about 1151 days (or about 3.2 years) for the slowly eliminated compound.

While these sorts of relationships are not typically used in any quantitative manner in deriving toxicity values, they may be useful in discussing temporal relationships in the dose-response assessment (Section 3.3). For many rapidly eliminated compounds, there is a very weak temporal relationship in dose-response and dose-severity relationships and these may sometimes be explained or at least rationalized using the plateau principle. Conversely, if large differences are noted in short-term and longer-term toxicity values for a rapidly eliminated chemical, this suggests that cumulative damage (i.e., damage for which the rate of repair is very slow rather than an accumulation of the chemical itself) may be occurring.

3.1.4. Acute Oral Toxicity

Acute oral toxicity studies are among the most commonly available types of information on chemicals. For pesticides, acute oral toxicity studies are typically available on the purified active ingredient, the technical grade active ingredient, and at least some formulations. If information is available on the toxicity of metabolites, this information is often limited to acute oral toxicity studies. Thus, these types of studies are often the only kind of information that is available to assess the toxicologic importance of inerts and adjuvants (Section 3.1.14) and of impurities and metabolites (Section 3.1.15).

Two types of acute oral toxicity studies are available: gavage exposure (e.g., OPPTS 870.1100) and dietary exposure (e.g., OPPTS 850-2400). Both types of studies involve administering

various doses or concentrations of the substance being tested to groups of test animals (typically rats) and observing the animals for a period of time after exposure (typically 14 days). Gavage studies are most common. In a gavage study, the test substance is administered by a stomach tube and a fixed amount of the material is placed into the stomach of the animal. For some chemicals, a vehicle must be used – i.e., the chemical is dissolved in a toxicologically inert compound such as water or corn oil prior to administration. In dietary studies, the compound is mixed into the normal diet of the animals (typically laboratory chow). Vehicles may also be used in dietary studies to facilitate the handling or mixing of the compound. Typically, at least five animals of each sex are used per dose or concentration and at least five doses or concentrations are used in a full study. The spacing between doses or concentrations is variable. In an oral gavage study, typical doses might be 1, 2, 4, 8, or 16 mg/kg/day in a closely spaced study. In a more widely spaced study, doses might be 1, 3, 10, 30, or 100 mg/kg/day. The idea behind the dose spacing is to obtain fractional mortality at several doses or concentrations. Without fractional mortality, statistical analyses of the data is limited (Section 3.3.5.).

Both gavage and dietary studies may involve either range finding studies or limit tests. Range-finding studies typically involve fewer numbers of animals and fewer but more widely spaced doses – often a factor of 10. Limit tests are conducted when it is suspected that a test substance is not very toxic. These are conducted at only a single dose or concentration. For the acute oral gavage study in rats used by OPP, the limit dose is 2000 mg/kg bw (U.S. EPA/OPPTS 2005, OPPTS 870.1100).

The results of acute toxicity studies are usually expressed as time-specific LD₅₀ or LC₅₀ values (doses or concentrations of a toxicant that result in 50% mortality of the test species during a specified exposure or observation period). The statistic methods used to compute these values are discussed in Section 3.3.5. In some studies, the results do not permit the calculation of LD₅₀ or LC₅₀ values. For example, if a limit test is conducted at a dose of 2000 mg/kg and no mortality is seen, no LD₅₀ can be calculated. In such a case, the results might be expressed as an LD₅₀ of >2000 mg/kg/day. This type of reporting can lead to confusion. For example, a full study in which the doses were 125, 250, 500, 1000, and 2000 mg/kg, and mortality was observed only at 2000 mg/kg/day and the response was 40% mortality could also express the results as an LD₅₀ of >2000 mg/kg/day. As discussed in greater detail in Appendix 2 (Guidelines for the preparation of appendices), an attempt is made to avoid this sort of ambiguity by reporting time-specific LD₅₀ or LC₅₀ values along with NOEL or NOEC values in addition to fractional mortality if appropriate. Thus, the two types of LC₅₀ values of >2000 mg/kg would be reported as either a NOEC for mortality of 2000 mg/kg or an LC₅₀ value of >2000 mg/kg with 40% mortality at 2000 mg/kg.

LD₅₀ and LC₅₀ studies may also involve oral, dermal, or inhalation exposures of mammals, birds, and some invertebrates like the honey bee. These types of studies are conceptually similar to the gavage LD₅₀ and dietary LC₅₀ studies conducted in this section and are discussed in lesser detail in other sections of this document as appropriate. All of these different types of data may be used to categorize the toxicity of a chemical. In terms of human health risk assessments, the

categories that may be discussed include those used by U.S. EPA for determining the use of signal words on labels that must accompany pesticide formulations (U.S. EPA/OPP 2003). These toxicity categories are summarized in Table 3-2 for oral and inhalation LC₅₀ values, dermal LD₅₀ values, and effects on the eye and skin.

For acute oral toxicity, the most toxic category is designated as Category I and involves compounds with oral LD₅₀ values of less than 50 mg/kg bw. The least toxic compounds fall into Category IV, defined as compounds for which LD₅₀ values are greater than 5000 mg/kg bw. These categories are used only in the hazard identification to reflect how the U.S. EPA would classify the chemical. As discussed in Section 1.2.1, the risk characterization must be based on both an exposure and a dose-response assessment. Toxicity categories for other endpoints are discussed below in the appropriate subsections and similar toxicity categories used in the ecological risk assessment are discussed in Section 4.1.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

3.1.5.1. General Considerations – Subchronic and chronic toxicity studies form the basis of most quantitative values used in risk assessments. The quantitative use of these types of studies typically involves identifying an NOEL (no observed effect level) or NOAEL (no observed adverse effect level) and dividing this value by one or more uncertainty factors. This quantitative use is detailed further in Section 3.3.2 (Chronic RfD). This section focuses on the kinds of data that are available and the factors that go into evaluating these data.

As with acute toxicity studies, the U.S. EPA (U.S. EPA/OPPTS 2005) has developed a number of very specific protocols and standards for subchronic and chronic toxicity studies. These include 28-day repeated dosing studies in rodents (OPPTS 870.3050), 90-day subchronic studies in rodents (OPPTS 870.3100) and non-rodents (OPPTS 870.3150), chronic toxicity studies in rodents (OPPTS 870.4100) and carcinogenicity studies in rodents (OPPTS 870.4200). For the 28-day and 90-day studies, the rodents are typically some strain of rat and the non-rodent assay typically involves beagle dogs. For the chronic rodent studies, both mice and rats are often included. While chronic toxicity studies and carcinogenicity studies have separate protocols and may sometimes be conducted separately, these studies are often combined into a single chronic toxicity/carcinogenicity study (OPPTS 870.4300). All of these studies usually entail some sort of preliminary range finding study, conceptually similar to the range finding studies conducted in acute toxicity studies. Chronic studies typically involve only 2 doses (although some may have up to 5 doses) with groups of about 50 animals per sex per dose. The subchronic studies will typically have a greater number of doses but may involve fewer numbers of animals per dose.

3.1.5.2. Statistical Considerations – While the number of doses and number of animals per dose level are not strictly defined, the criteria for an *acceptable study*, at least in terms of acceptability for pesticide registration, is that the study identifies both an NOEL or NOAEL as well as an adverse effect level (LOAEL). The method of determining an NOAEL or LOAEL typically involves comparisons between the control group and one of the dose groups. A commonly used statistical test is called the Fisher Exact Test which is used to determine if the incidence in a dose

group is significantly higher than the incidence in the control group (Uitenbroek 1997). As a convention, a significance level of 0.05 (often expressed as $p < 0.05$) is used as a criterion for statistical significance. This level of significance indicates that the probability that the difference in incidence between the control group and the dose group occurred by chance is less than 5 percent.

In some cases, multiple comparisons must be made – i.e., the incidence of several different effects between dose groups. In dealing with multiple comparisons, however, the use of the standard p -value of 0.05 may overestimate the number of significant associations. For example, if 100 sets of comparisons are made within the same population—i.e., there are by definition no differences because there is only one population—some comparisons may appear to be statistically significant only because of random differences in the sampling. To address this issue, one standard approach is to divide the pre-determined significance level, typically taken as 0.05, by the number of comparisons being made. This is referred to as Bonferroni's correction (e.g., Curtin and Schulz 1998). A large number of other statistical methods – ranging from common t -tests to more sophisticated methods to determine whether a dose-response relationship is significant – may be used. These are discussed and referenced as necessary in each risk assessment.

3.1.5.3. Definition of Adversity – While statistical considerations are important in defining whether or not a particular effect is associated with a particular level of exposure, statistics do not address whether or not a particular effect should or should not be classified as adverse. Central to any risk assessment is the interpretation of the effects that are seen and the classification of these effects as incidental, adaptive, or adverse.

The identification of adverse health effects is, in some cases, trivial. Death is obviously an adverse health effect. In other cases, however, the classification of a particular effect as adverse may involve objective analysis, professional judgment, and even subjective or ethical considerations. For example, an extreme position would be that any effect caused by a chemical is adverse. At the other extreme is the position that only gross and obvious signs of toxicity should be regarded as adverse. Most organizations involved in risk assessments have adopted, at least implicitly, a central position. Some effects are classified as *adverse* and others are not, and judgments are usually based on the clinical significance of the magnitude or intensity of an adverse effect.

In general, any deviation from the state of health can be considered an adverse effect. For the purposes of defining an adverse health effect, the definition of health can be given as:

health - *the condition of an organism or one of its parts in which it performs its functions at a level which is at least adequate for the normal activity of the whole organism over the normal lifetime of the organism.*

The term health may be applied either to the whole organism or its component parts. This definition explicitly incorporates the relative nature of health, which can be described by three categories—adequate, normal, and optimal—recognizing that these are general states in a graded series. These categories are intended to have a clinical interpretation in terms of the severity of the effect. A deviation from *adequate health* would constitute an obvious and frank disease state. At the other extreme, a deviation from *optimal health* would be characterized as a detectable and statistically significant decrement in function with the magnitude of the decrement remaining within the normal range of clinical variation. A deviation from *normal health* would be considered as any decrement in function outside the normal clinical range that does not result in a frank disease state.

The whole organism must be able to function in at least a normal manner. At any lower level of observation, a sub-normal functioning is classified as *unhealthy* to the extent that the reduction in normal activity of the subsystem is affected.

Based on this definition of health, an adverse effect may be defined as:

adverse effect - any effect that decreases the capacity of an organism or a component of the organism to function in a normal manner, or that leads to a frank disease state.

This definition intentionally omits any distinction between signs (objective and measurable indices of an effect) and symptoms (subjective reports) of toxicity. Subjective reports of effects such as headache, nausea, and dizziness often must be addressed in a risk assessment. In some instances, these symptoms may be associated with anxiety rather than exposure. In assessing the toxicity of an individual chemical, the issue of attribution is critical, but this does not affect the definition of an adverse effect. If the symptom is consistent with the above definition of an adverse effect, then the symptom is considered an adverse effect whether or not it is attributable to chemical exposure.

Another very explicit and intentional limitation of this definition is that it does not consider **severity** and does not limit the definition of an adverse effect to any particular level of observation. In other words, any effect on the function of the whole organism or any of its component parts at any biological level is considered adverse.

For example, at the molecular level, the formation of methemoglobin incapacitates the hemoglobin molecule just as the reaction of organophosphates or carbamates inactivates acetylcholinesterase. Thus, the formation of methemoglobin or the inhibition of an enzyme such as acetylcholinesterase is regarded as an adverse effect regardless of the magnitude of the effect because such interactions reduce the ability of the organism to respond to further stress (i.e., agents that induce methemoglobin or inhibit acetylcholinesterase).

Structural alterations, albeit secondary to functional changes, can be an indicator of impairment. The relationship between structural and functional effects is not always clear. As detailed by

Ruben and Rousseaux (1991), some pathological changes are clear indices of functional impairment while other changes may have a more complex interpretation or be of questionable toxicological significance. Consequently, general guidelines for classifying a particular type of lesion as adverse or not adverse cannot be given. As detailed in the companion report, the significance of a pathological lesion depends on the nature and site of the lesion as well as other information regarding the health of the organism.

3.1.5.4. Some Key Endpoints – NOAEL or LOAEL values may be based on virtually any organ or organ system in the body. Some effects are of central concern for a variety of reasons. Such effects include effects on the nervous system, immune function, the endocrine system, reproductive and teratogenic effects, and carcinogenic and mutagenic effects. Each of these categories of effects are discussed in subsequent subsections of this document and are included as separate and corresponding subsections in each risk assessment. Nonetheless, there are a larger number of other organs or organ systems that may not be linked directly to any of these effects of special concern. While it is beyond the scope of this document to cover all effects that might be observed, two classes of effects – effects on body weight or organ weight and effects on the liver – are discussed below.

Body and Organ Weight : All organisms grow and develop in regular patterns. Normal body weights and organ weights for humans have been relatively well documented (Snyder et al. 1975), and similar data are available on a variety of other species (e.g., Altman and Dittmer 1972 and 1974; U.S. EPA 1989a,b,1993). Furthermore, a variety of methods have been developed to quantify and model animal growth and development (e.g. Karlberg 1987; Moore 1985).

There is no doubt that changes in growth rate (i.e., whole body weight) and organ weight associated with chemical exposure can be indices of toxicity. For example, dioxins cause a wasting syndrome in experimental animals that is at least partially characterized by a general decrease in body weight. Similarly, diuretics or uncouplers of oxidative phosphorylation can cause general decreases in body weight. For all of these compounds, the gross decrease in body weight can be directly related to the mechanisms of action of these compounds.

At the level of the organ, decreases or increases in weight may also be related to mechanisms of action. Perhaps the best studied example is liver enlargement associated with the induction of mixed function oxidases (MFO). Many compounds that induce MFO may cause a corresponding and profound increase in liver weight that can be related directly to the mechanism of action of these compounds. In some cases organ weight changes may reflect clear toxicological processes. For example, compounds that damage the lungs may cause edema, which will be reflected as an increase in absolute or relative lung weights. Toxicological damage also may be reflected as a decrease in organ weight, such as the effects of some phthalates on the testicles.

In all of these examples the interpretation of the significance of changes in whole body or organ weights is linked to an understanding of how the particular chemical influences the organism or organ. Notwithstanding these examples, changes in whole body weight or organ weights, in the

absence of any information on the mechanism of action or clear signs of toxicity, are marginal kinds of information for classifying a particular exposure as adverse or not adverse. Nonetheless, these effects may be used by groups such as the U.S. EPA as a conservative/protective basis for defining an NOAEL and it is not uncommon for RfD's and similar values to be based solely on changes in body weight or organ weight in the absence of data indicating that these effects are not toxicologically significant. If a decrease in whole body weight can be related to a decrease in food consumption, the effect may simply reflect an organoleptic property (i.e., the compound may make the food unpalatable to the organism). A decrease in food consumption, however, may also reflect an underlying pathology that suppresses the appetite of the organism. Thus, a decrease in whole body weight associated with a decrease in food consumption does not necessarily indicate the absence of an adverse effect.

Liver : The liver is an extremely common target tissue in toxicology and is discussed in detail in standard toxicology texts (e.g., Moslen 1996); effects on the liver commonly serve as a basis for many RfDs derived by the U.S. EPA. The liver is important not only in the activation or detoxication of xenobiotics but also in various normal physiological functions, including several functions affecting the blood (clotting factors) and the metabolism, synthesis, and/or regulation of a variety of endogenous substance, such as various sugars, fatty acids and fat catabolism and anabolism, amino acid and protein synthesis, the removal of toxic metabolites such as ammonia, the storage of iron and copper, the synthesis of vitamin A and the storage of vitamins A, D, and B₁₂, and the secretion of bile into the gastrointestinal tract via the gall bladder.

At the level of the whole organism, one of the most common effects of liver dysfunction is jaundice, the accumulation of bilirubin and other bile pigments in the skin and mucous membranes, which is associated with obstruction of the bile ducts (*obstructive jaundice*). Jaundice may also be caused by an increase in the destruction of red cells by the spleen (*hemolytic jaundice*). Because of the importance of the liver in a variety of metabolic processes, liver damage can lead to a great variety of other gross toxic effects.

When liver cells are damaged, various enzymes may be released into the plasma, depending on the test species and the type of damage, and may serve as very sensitive measures of liver damage. The interpretation of changes in plasma enzymes, however, is complicated by the wide tissue distribution of some enzymes as well as varying rates of enzyme synthesis and plasma clearance. Because the plasma clearance rates for most enzymes are relatively rapid, plasma enzyme levels are more commonly used in acute rather than chronic studies. Commonly used enzymes for assessing hepatocellular damage include aspartate and alanine aminotransferases as well as lactate, sorbitol, malate, and glutamate dehydrogenases (Woodman 1988). In the absence of any other signs of liver toxicity, increases in plasma levels of these enzymes may be used to classify an effect level – i.e., a dose or concentration – as adverse.

In contrast to plasma levels of liver enzymes, changes in plasma levels of endogenous proteins, which are synthesized by the liver, are generally indicators of more severe damage because they more closely reflect functional impairment. Effects on endogenous proteins include changes in

serum albumin, fibrinogen, α_1 antitrypsin, haptoglobin, ceruloplasmin, transferrin, and prothrombin (Woodman 1988). Because of the longer protein half lives and slower clearance rates of plasma proteins compared with plasma enzymes, these changes are more likely to reflect chronic damage.

Changes in plasma lipid levels usually indicate more serious effects on liver function (Woodman 1988). Increased plasma concentrations of cholesterol, low density lipoproteins, triglycerides, non-esterified fatty acids, and phospholipids, while not specific to liver damage, are suggestive of serious functional impairment. If other more specific data (e.g., effects discussed above or fatty accumulation in hepatocytes) indicate that such changes in plasma lipids are likely to be associated with liver damage, these effects should be classified as less serious or more serious but not as minimal.

One of the most common histopathological changes observed in the liver is necrosis or cell death. Classically, liver necrosis is characterized as massive, focal, centrolobular, midzonal, and periportal (Popp and Cattley 1991). As its name implies, massive necrosis involves large portions of one or more lobes of the liver and is readily apparent on gross necropsy. Because of the large reserve capacity of the liver, massive necrosis does not necessarily lead to death, and lobules with enough viable hepatocytes may regenerate. In other words, massive necrosis can be reversible. In more severe cases, massive necrosis will lead to fibrosis, with relatively few viable hepatocytes in the lobe. In spite of the potential reversibility of this condition, massive necrosis clearly indicates a substantial decrement in organ function, which may be fatal or debilitating. At the other extreme, focal necrosis consists of relatively small (<1 mm) areas of dead hepatocytes. Although the pathogenesis of focal necrosis is poorly understood, this condition is reversible, does not lead to fibrosis, and is observed in animals that do not show clear signs of toxicity. Although this condition is clearly adverse at the cellular level, it is not clearly associated with impaired liver function.

Centrolobular, midzonal, and periportal necrosis are intermediate and variable in severity. It is difficult to generalize the level of adversity on the basis of these designations alone. Centrolobular necrosis associated with hemorrhagic lesions or involving endothelial cells may be indicative of a more serious adverse effect. Otherwise, the rapid reversibility of this condition, usually 1 week, suggests that this is a less serious adverse effect (i.e., it may have a transient effect on organ function but is not likely to lead to frank signs of toxicity). Periportal and midzonal necrosis are less common than centrolobular necrosis but are also rapidly reversible.

As discussed above, liver damage may result in increased lipid synthesis, which can lead to a condition referred to as fatty liver, fatty degeneration, or lipidosis. Moderate fatty changes do not seem to affect organ function. Other common forms of hepatocytic pathology include hydropic degeneration, glycogen accumulation, accumulation of lipofuscin pigment, and excess storage of iron and copper. All of these changes are reversible, and, in the absence of other signs of liver toxicity, are not usually associated with organ dysfunction. Thus, although these changes are classified as adverse effects, they are adverse at the cellular level but do not substantially impair

the function of the organ or whole animal. Several other lesions in nonparenchymal cells or bile ducts may be associated with chemical exposure. Hepatitis and cirrhosis, like massive necrosis, can lead to profound clinical signs of toxicity and are clearly adverse effects.

3.1.5.5. Epidemiology Studies – Strictly defined, epidemiology refers to the study of disease patterns in humans. When good epidemiology data are available, they can serve as the definitive qualitative and/or quantitative estimate of potential human hazard. Occasionally, the unique nature of a chemically-induced effect (e.g., liver angiosarcoma by vinyl chloride) will lead quickly to the recognition of a human risk. More often, however, epidemiology studies in non-occupational situations are not definitive enough to establish chemical cause-and-effect relationships with certainty.

For some compounds, information may be available on toxic effects associated with accidental or normal occupational exposures. This type of information may be used to assess dose-response relationships in humans. Data from human exposure incidents must be carefully analyzed with regard to the nature of the chemical involved, the quantity of chemical present, and the duration of exposure. In addition, the possibility of synergistic and antagonistic effects of other chemicals is quite significant, especially in industrial situations. It is hoped that reports of human surveillance studies, including personal monitoring data as well as retrospective investigations in work populations, can be obtained. Because human living conditions and lifestyles vary greatly, a detailed analysis of particular human situations involving chemical exposures can be extremely valuable in defining public health hazards. Furthermore, toxicological screens and animal model systems cannot substitute for every aspect of human living conditions and cannot duplicate the everyday exposures to which humans are subject.

Information regarding the human health effects of chemical exposure should come from human experience; however, these data are difficult to obtain. Controlled laboratory experiments in which humans are exposed to chemical substances are limited by ethical considerations. When chemicals are administered to humans under controlled conditions, the results may be inconclusive because of inter-individual variability and because of the generally small number of individuals participating in the studies. Case reports of persons with known exposure to a particular chemical generally provide qualitative evidence of a causal relationship between exposure to that chemical and a particular toxic effect, but exposure levels are seldom known and control data are not available.

Epidemiology studies are often difficult to interpret and open to criticism because these studies are very difficult to control; it is difficult to identify two virtually identical groups of individuals for which the only significant or substantial difference is exposure to the chemical. In addition, most epidemiology studies do not well-characterize exposure and the exposures themselves are not controlled. For these reasons, it is unusual for epidemiology studies to serve as the sole basis for a hazard identification, and it is very unusual for epidemiology studies to serve as the basis for a dose-response assessment. This is similar to the use of field studies in ecological risk

assessment (Section 4.1). Epidemiology data are most often used in combination with standard toxicity studies in laboratory animals to assess whether or not a particular effect is plausible.

3.1.6. Effects on Nervous System

The EPA/OPPTS (2005) does have protocols for some specialized studies on effects on the nervous system. These include a general neurotoxicity screening battery (OPPTS 870.6200), an acute and a 28-day delayed neurotoxicity assay for organophosphorus substances (OPPTS 870.6100), a developmental neurotoxicity assay (OPPTS 870.6300), and an assay for peripheral nerve function (OPPTS 870.6850). It should be noted that the delayed neurotoxicity study (OPPTS 870.6100) uses hens (*Gallus gallus domesticus*) as a test species. For this particular assay, this is considered a sensitive species that is relevant to the assessment of neurotoxicity in humans.

For most pesticides, specific studies designed to detect neurotoxicity are conducted only if neurological effects are noted in more routine toxicity studies, or if the chemical belongs to a class for which there is a strong presumption that all members of the class will be neurotoxic. Typical subchronic or chronic animal bioassays rely on morphological and functional assessments to detect neurotoxicity. Morphological assessment usually consists of examination of the brain and spinal cord for visible changes at the naked-eye and light microscopic level. Structure of the terminal portions of the peripheral nervous system is evaluated as part of the morphological examination of endocrine and exocrine glands, muscles, and other tissues. In some assays, including the standard procedures used by the National Toxicology Program (NTP), evaluation of the spinal cord and peripheral nerves (e.g., sciatic nerve) is only performed if the study finds other indications of neurotoxicity.

Behavioral assessments typically include observations of the animals in their cages for gross deficits in movement, balance, or coordination (e.g., gait, posture, visible tremor) (O'Donoghue 1996). These are sometimes further supplemented with a more comprehensive functional observation battery consisting of various qualitative or quantitative tests of movement, gait, balance and coordination, muscle strength, and reflexes (Weiss 1999). Beyond the realm of most typical bioassays are various, more explicit tests of motor, sensory and cognitive function that can provide a more quantitative evaluation of neurological deficits (Weiss 1999). These would usually be conducted only if there were other indications of a possible direct neurotoxic effect of the agent.

Assays for neurological effects may be complicated and difficult to interpret because of the complexity of the nervous system itself. The nervous system can be subdivided anatomically into the *central nervous system* (CNS), which includes the brain and spinal cord, and the *peripheral nervous system* (PNS), which includes nerves connecting organs and tissues with the spinal cord and brain. The latter include the nerves that carry information to the CNS about sensation (*sensory neurons*), and nerves that carry information to muscles to control movement (*motor neurons*). From the perspective of mechanisms of neurotoxicity, the nervous system can be more meaningfully subdivided into the various functional components of nerve cells

(*neurons*) that can be the targets of chemical agents. The structural organization of neurons reflects their principal function to process, store, and convey information about the body, either within the CNS, or between the CNS and other tissues and organs. This is accomplished by a combination of chemical signaling between neurons, and electrical potentials and currents within neurons. Neurons consist of 1) a *cell body*, containing the nucleus and other organelles that carry out synthesis and catabolism; 2) *dendrites*, elongated cellular processes that emanate from the cell body and that function to receive information, in the form of chemical signals, from other neurons and translate these signals into electrical potentials and currents within the cell body; 3) the *axon*, an elongated process (which can be more than a meter in length) that transmits information, in the form of electrical potentials and currents, from the cell body to nerve terminals; and 4) the *nerve terminal* which receives information encoded in electrical currents from the axon and communicates, in the form of chemical signals, to other neurons. In addition to neurons, the nervous system includes a variety of other types of cells that are critical to the function of the system. These include neuroglia (in the CNS), Schwann cells (in the PNS), and various specialized sensory receptors (in the PNS). Neuroglia and other supporting cells make up approximately 90% of the cells in the CNS (Jones 1988).

Neurotoxicants are chemical agents that disrupt the function of neurons, either by interacting with neurons specifically, or with supporting cells in the nervous system (e.g., neuroglia, Schwann cells, sensory receptors). The above definition is central to this discussion because it distinguishes agents that act directly on the nervous system (*direct neurotoxicants*), from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*) (O'Donoghue 1994). An example of the latter would be an agent that disrupts the respiratory or cardiovascular system and, thereby, deprives the brain of oxygenated blood. Another example would be an agent that disrupts kidney function and, thereby, alters nervous system function by producing irregularities in body sodium and potassium levels.

The term *indirect neurotoxicants* may be a misnomer – or at least somewhat confusing – in that the indirect agent, by definition, does not directly damage nerve tissue. Nonetheless, the distinction between direct and indirect neurotoxicants is important because the types of biological assays needed to fully characterize these two very different causes of neurological effects will be very different. For example, an agent that disrupts kidney function may also produce, secondarily, neurological effects that are similar to those produced by agents that disrupt potassium or sodium transport in nerve cells (lethargy, stupor, muscle tremors, convulsions). However, in a typical whole animal chronic toxicity bioassay, such effects would be observed in concert with irregular serum sodium and potassium levels, and other indications of impaired kidney function. The same neurologic effects observed in the absence of indicators of impaired kidney function, or impaired function of other organ systems that might secondarily result in neurological effects, would be much more provocative evidence that the agent might be a direct neurotoxicant. However, bioassays directed at detecting specific forms and mechanisms of neurotoxic activity would be needed to confirm that the agent is a direct neurotoxicant. These might include evaluations of motor or sensory function, histopathological examination of the nervous system for assessment of exposure-related structural changes, or assessments of the

toxicity of the agent in *in vitro* preparations of neurons of nervous system cells (these assays are described in greater detail below). In general, lethal exposures to toxic agents, regardless of their mechanism of toxicity, almost always give rise to neurological effects in the terminal stages of the intoxication. These effects can arise from many causes, including fluid and electrolyte imbalances, pulmonary edema, or cardiovascular collapse. Thus, very little information about the direct actions of a chemical agent on the nervous system is typically gained from studies of acute lethal, or near-lethal intoxications. The assessment of direct neurotoxic potential usually relies on studies of subchronic, chronic, or acute exposures, well below those that produce effects on other organ systems that might imperil the nervous system.

Evidence of neurotoxicity relies largely on the corroborated demonstration, usually in animal models, of a dose-related abnormality in the structure (*morphology*) of the nervous system (*histopathologic change*) and/or a dose-related effect of the chemical on neurologic function, such as impaired movement, response to sensory stimuli, learning, or memory. The occurrence of both histopathologic changes and functional deficits, in particular if the histopathologic changes occur in regions of the nervous system thought to control the observed function, would be strong evidence for neurotoxicity.

3.1.7. Effects on Immune System

The *immune system* consists of a set of first defense agents including the mononuclear phagocytic cells (macrophages in tissues, monocytes in circulation) and the natural killer cells, as well as specific lymphoid tissues dispersed throughout the body. The *lymphoid tissue* is comprised of T and B lymphocytes, epithelial cells and stromal cells, and is arranged into structurally and functionally distinct organs such as the *thymus*, *spleen*, and *lymph nodes* or accumulations of diffuse lymphoid tissue such as the *gut-associated lymphoid tissue* (GALT). All cells of the immune system derive from a pluripotent stem cell in the *bone marrow*. *T lymphocytes* become immunologically competent (mature) in the thymus. *B lymphocytes* mature in the GALT (mammals) or bursa of Fabricius (birds). The immune system defends its host against foreign agents by utilizing both the non-specific and specific components, its mature lymphocytes with their associated cell-surface antigens (cellular immunity), special proteins in circulation (immunoglobulins), specific *antibodies* produced by the plasma cell (humoral immunity) in response to foreign *antigens* (bacterial, viral, parasites, foreign proteins etc.) and a number of other cell products known as *cytokines*. Cells of an individual are recognized as self by their cell-surface recognition antigens. Each individual has a unique signature of cell recognition antigens, known as the *major histocompatibility complex* (MHC). Changes in these signature antigens identifies a cell as foreign or abnormal, and triggers an unwanted immune response. The MHC together with other types of cell-surface antigens on lymphocytes (cluster differentiation antigens, CD) enable the immune system to recognize and respond to foreign or abnormal cells. In *autoimmune* diseases, this recognition system fails, and the immune system mounts an often destructive response against self cells and tissues. Examples of autoimmunity include Hashimoto's thyroiditis due to the production of antibodies to native thyroglobulin, which is the major iodine-containing protein; autoimmune haemolytic anaemias, in which patients produce antibodies to their own red cells, and the Goodpasture's syndrome in which autoantibodies are

produced to glomerular basement membrane of the kidneys leading to glomerulonephritis (kidney damage).

Immunotoxicants are chemicals that disrupt the function of the immune system. Depending on the mechanism of action, immunotoxicants can either impair immune responses (immune suppression) or stimulate the immune responses (hyperreactivity). *Immune suppression* may lead to enhanced susceptibility to infectious agents or inability to clear cancerous cells from the system. Examples of immunotoxicants include corticosteroids, which are drugs used in the treatment of inflammation, and cyclosporin, a drug used to suppress the immune response in transplantation patients (Diasio and LoBuglio 1996). Environmental pollutants that are known to be immunosuppressive include benzene, PAHs, PCBs, TCDDs, certain heavy metals (e.g. lead, mercury and cadmium), and certain organophosphate and organochlorine insecticides (Burns et al. 1996; Luster et al. 1992, 1993; Tryphonas and Feeley, 2001). *Hyperreactivity* can lead to *allergy or hypersensitivity* in which the immune system of genetically predisposed individuals responds in an exaggerated manner to substances (allergens) such as plant pollen, cat dander, peanuts, and eggs, that do not pose a threat to other non-susceptible individuals. This type of reaction involves a sensitization phase during which the individual is subjected to repeated exposures of the allergen and a subsequent encounter with the allergen which may result in a mild reaction (skin rashes or hives, congestion, sneezing etc) or a less frequent but severe reaction (*anaphylaxis*) leading to death. Hyperreactivity can also lead to autoimmunity in which the immune system produces antibodies to self antigens resulting in damage of the organ or tissue involved. Only a few agents, mostly metals, have been shown to cause autoimmunity.

Evidence of immunotoxicity relies largely on the corroborated demonstration, usually in animal models, of a dose-related histopathologic change in lymphoid tissue and/or a dose-related effect of the chemical on immune response to a foreign antigen. The occurrence of both histopathologic changes in lymphoid tissue and abnormalities in one or more types of immune responses, would be strong evidence for immunotoxicity. Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (thymus weight is usually measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

The U.S. EPA (U.S. EPA/OPPTS 2005) has only one specific protocol for an assay of immune function, OPPTS 870.7800. This is a 28-day bioassay in which rats or mice are exposed to the test chemical. Typically, exposures are conducted by the oral route although dermal and inhalation routes can be considered for some compounds. The test assays response to a T cell dependent antigen, sheep red blood cells (SRBC). The animals are exposed to SRBCs about four days before the exposure period ends, after which assays are conducted for splenic anti-SRBC (IgM) response and/or serum anti-SRBC IgM levels. If significant depression in response to the antigen is noted, additional assays for immune function are required.

A variety of other tests have been developed to assess the effects of chemical exposures on various types of immune responses (e.g., Luster et al. 1988, 1992, 1993). These include measuring the effects of chemical exposure on antibody-antigen reactions (*humoral immunity*), measuring changes in the activity of specific types of lymphoid cells when exposed to foreign antigens (*cell-mediated immunity*), and assessing changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells (*host resistance*). Tests of immune responsiveness are not typically conducted as part of standard toxicity bioassays, unless there are other indications that the chemical may have immunologic potential. These indications might include histopathologic change in lymphoid tissue, changes in blood leukocyte counts, or indications of excessive infectious disease in treatment groups.

3.1.8. Effects on Endocrine System

A variety of short-term *in vitro* and *in vivo* tests have also been described that assess whether a chemical interferes with hormone availability (e.g., synthesis, secretion, transport in the bloodstream) or with the target tissue response (e.g., hormone receptor binding or postreceptor processing). These assays can be used to assess the potential for endocrine disruption and have been proposed as screening assays for endocrine disruption (EDSTAC 1998). The observation of endocrine activity of a test chemical in these short-term assays together with the observation of abnormalities in growth, development, reproduction, homeostasis, or in endocrine glands, in a multigeneration study in whole animals, would be strong evidence that a chemical is a potential endocrine disruptor. The U.S. EPA/OPPTS (2005) has not yet recommended a specific assay or battery of assays for endocrine disruption.

The *endocrine system* is critical to the health of an animal because it participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands*, *hormones*, and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. *Hormones* are chemicals produced in endocrine glands that bind to *hormone receptors* in target tissues. Binding of a hormone to its receptor results in a process known as *postreceptor activation* which gives rise to a *hormone response* in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone *testosterone* from the male testis, or *estrogen* from the female ovary, which act on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion. An example of this would be the hormone LH (luteinizing hormone), secreted from the pituitary gland, which acts on receptors in the testis to stimulate the secretion of testosterone. This system of endocrine glands, that are responsive to hormones released from other endocrine glands, provides a complex network of control systems for turning on and turning off hormone stimulation of tissues in response to physiological demands, or at

appropriate stages of the life span or reproductive cycle. Examples of this are the dramatic changes in growth and development that occur as the fetus develops in the uterus and as individuals sexually mature during puberty. Repeated cycles of turning on and turning off hormone stimulation of the ovary and uterus occur approximately each month in females to produce the menstrual cycle.

An *endocrine disruptor* is an exogenous agent (from outside of the body) that produces adverse effects on an organism or population of organisms by interfering with endocrine function (Kavlock et al.1996). The endocrine system is highly regulated to achieve hormone activities in amounts needed to respond to physiological demands. *Endocrine disruption* is a state of uncontrolled hormone action, in which hormone responses are absent or insufficient when needed, or occur inappropriately when they are not needed. These can result in abnormalities in growth and development, reproduction, body composition, homeostasis, and behavior. Endocrine disruptors are not considered to be a major cause of endocrine disorders in humans. However, a variety of inherited endocrine diseases are known to be caused by abnormalities in endocrine glands, hormone transport, or hormone receptors. Certain endocrine diseases are thought to be caused by autoimmune disorders in which the body attacks and destroys its own endocrine glands.

Some important drugs are endocrine disruptors. Examples of these include thyroid blocking agents used in the treatment of hyperthyroidism (e.g., thiopropyluracil); corticosteroids used in the treatment of inflammation, and as diuretics in the treatment of edema and hypertension; estrogens used in female birth control and to manage symptoms of menopause; hypoglycemics used in the treatment of certain forms of diabetes mellitus; and various adrenergic agonists and antagonists used in the treatment of allergic reactions, asthma, heart disease, and hypertension (Hardman and Limbird1996). Endocrine-active agents are also in our diet, including iodine, needed for the production of thyroid hormone, and phytoestrogens, estrogenic compounds found in many edible plants.

Endocrine disruptors can exert effects by affecting the availability of a hormone to its target tissue(s) and/or affecting the response of target tissues to the hormone (EDSTAC1998). These effects can enhance the action of natural hormones or diminish or abolish these actions. Effects may be transient or permanent, and may occur soon after exposure to the agent or may occur long after exposure ceases (*latent*).

Evidence of endocrine disruption relies on the corroborated demonstration, usually in animal models, of a dose-related abnormality in the structure of endocrine glands (histopathologic change); a dose-related effect of the chemical on endocrine function, including hormone synthesis, secretion, transport and elimination; receptor binding; or postreceptor processes that give rise to a response in a target tissue and a demonstration that the effect on endocrine function gives rise to an adverse effect in the organism or population (EDSTAC1998). Examples of adverse effects include impairment in growth or development, reproduction, homeostasis, or behavior. This latter evidence of an adverse effect is particularly important, since it distinguishes

endocrine disruptors from chemicals that are merely endocrine-active but have little or no potential for disruption of the endocrine system.

Morphological examination of the major endocrine glands for histopathologic changes are usually included in well-designed subchronic or chronic rodent bioassays. However, typical rodent subchronic or chronic bioassays begin exposures after weaning, whereas, the assessment of potential adverse consequences of endocrine disruption requires the evaluation of exposures that span all of the critical stages of the lifespan at which endocrine controlled growth and development occur (EDSTAC1998). Organisms may be particularly sensitive to endocrine disruption during embryonic development and post-natal, and during growth and maturation (e.g., puberty). Disruption of the endocrine system during development may give rise to effects on the reproductive system that may be expressed only after maturation. For this reason, multigeneration exposures are recommended for toxicological assessment of suspected endocrine disruptors (EDSTAC 1998). These assays are discussed further below (Section 3.1.9).

Dose-response relationships for endocrine disruptors may be complex; the response may increase or decrease over intervals of a dose range of a given agent. For example, testosterone can stimulate sperm production at low doses and inhibit sperm production at high doses (EDSTAC1998). As a result, assays conducted at a high dose range may not be predictive of responses at a lower dose. Dose ranging studies are recommended to ensure that the assays include a dose range of adequate width to include a clearly toxic dose (maximum tolerated dose) and to capture possible *low-dose effects*. If these types of assays examine an adequately wide dose range below and including the maximum tolerated dose, they can be expected to detect adverse consequences, including latent consequences, of endocrine disruption. However, they cannot be expected to provide definitive conclusions about whether the observed abnormalities do in fact result from endocrine disruption. Other studies directed at identifying endocrine mechanisms underlying the abnormalities would be needed for this purpose.

3.1.9. Developmental (Teratogenic) and Reproductive Effects

3.1.9. 1. Developmental (Teratology) Studies – Chemically-induced reproductive impairment is an important response parameter in human and ecological risk assessments. In human risk assessment, teratogenicity, sterility, or decreased reproductive capacity can serve as endpoints in establishing NOELs from chronic exposure. However, the threshold for adverse reproductive effects in mammals is often above the threshold for more general toxic effects (e.g., decreased total body weight gain or altered organ weights). Furthermore, many mammalian teratology studies involve single short-term exposures and are difficult to apply directly to estimating the risk from environmental exposure.

Teratogenicity studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are typically required for the registration of pesticides. Protocols for developmental studies have been established by U.S. EPA/OPPTS (2005) – i.e., OPPTS 870-3500 and 870.3700. Typically, the compound is administered by gavage and at least three doses are used;

exposure may occasionally be dietary or inhalation. The compound is administered daily to pregnant female animals over a specified period during gestation. The dams are observed for signs of toxicity and the offspring are observed for signs of abnormal development. Other endpoints that are examined include signs of pre-implantation losses and resorptions.

Developmental toxicity relates specifically to effects on the embryo or fetus and not to the pregnant female. Although this area of study was traditionally concerned with compounds that resulted in the birth of grossly abnormal offspring, it has been expanded to encompass those dose-related effects resulting in death of the embryo or fetus, functional impairment and altered growth and/or developmental patterns. The physiological processes that produce abnormal development are the same cellular mechanisms associated with chemical toxicity in the adult, including inflammation, degeneration, necrosis, cell differentiation, and proliferation. Nonetheless, the embryo and fetus are viewed as a uniquely susceptible target, due to the occurrence of unusually rapid proliferation and differentiation during fetal development.

Teratology studies may occasionally be used to derive acute RfDs (Section 3.3.3). The rationale for this use is that most teratology studies involve relatively short-term exposures. In addition, many teratogenic effects are very time-specific and it is possible that only a single exposure or dose occurring on a single critical day during development could account for the observed effect.

3.1.9.2. Multigeneration Reproduction Studies – Another type of reproduction study involves exposing one or more generations of a test animal to a compound. Protocols for reproduction studies have been established by U.S. EPA/OPPTS (2005) – i.e., OPPTS 870-3800. Typically, the compound is administered at 3 or more dose levels. Dietary administration is the most common route although drinking water or gavage studies are sometimes conducted. These tests are almost always conducted on rats. The general experimental method involves dosing the parental (P) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to, during mating, after mating, and through weaning of the offspring (F1). Typically, this procedure is repeated with male and female offspring from the F1 generation to produce another set of offspring (F2). During the study, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number and viability of offspring.

This is a very important type of study for many risk assessments. As discussed in Section 3.1.8, multigeneration reproduction studies are often the most relevant of the commonly available studies for assessing the effects of a compound on endocrine effects relating to reproduction. In addition, multigeneration reproduction studies may be used by U.S. EPA to derive acute RfDs (Section 3.3.3). As with teratology studies, the rationale for this use is that adverse effects on reproduction may be linked to very brief periods during development.

3.1.9.3. Target Organ Toxicity – As part of most standard acute and chronic toxicity studies, observations are often made on reproductive tissue – e.g., ovaries and testes. This type of information is often included in this section (and may be repeated from previous sections for

emphasis). This type of information can be used to assess concern for potential reproductive effects and to supplement information from developmental or reproduction studies.

3.1.10. Carcinogenicity and Mutagenicity

Three kinds of data are commonly used to assess potential carcinogenic hazard: epidemiology studies; bioassays on mammals; and tests for genetic toxicity, including mutagenicity. The general protocols for carcinogenicity studies are similar to those of chronic toxicity studies and are discussed in Section 3.1.5.1.

Epidemiology studies involve the comparison of the cancer incidence in two or more populations with varying degrees of exposure to the chemical under study. They are of limited use because many important variables—like quantitative estimates of exposure to the test chemical, differences in diet, and exposure to other potential carcinogens—are not adequately controlled or characterized. Nevertheless, data from well-designed epidemiology studies are the only data accepted as unequivocal proof that a chemical is a human carcinogen. The problems in precisely defining exposure levels and other factors merely inhibit the use of these studies in quantitative risk assessment.

Bioassays on mammals involve the controlled exposure of experimental animals, usually rats or mice, to defined levels of the test substance. In carcinogenicity bioassays, an attempt is made to expose the organism for a significant portion of its life span to the test substances, or at least to observe the organism for a significant portion of its life span. This protocol is necessary because many tumors appear only late in the life of the organism; thus, premature sacrifice may lead to false negative results. Furthermore, in terms of environmental toxicology, the major concern is the incremental increase in the incidence of cancer attributable to lifetime exposures. Another important element in the design of mammalian bioassays is the proper selection of dose levels. Since for practical and economic reasons, only limited numbers of animals (usually 20-50) are used at each dose level, it is necessary to use elevated dose levels in order to elicit a detectable response. Because excessively high doses that result in overt signs of toxicity may alter the physiology of the animal so that it is no longer a reasonable model for projected human exposures, attempts are made to ensure that doses below the maximum tolerated level are used. In addition, excessively high doses can cause premature mortality, which may mask carcinogenic activity. At the end of the experimental period, all animals are sacrificed and subjected to extensive histopathological analyses. A positive response is usually defined as a significant dose-related increase in the incidence of malignant tumors at a given site in exposed animals.

Because carcinogenicity bioassays are time consuming and expensive, it is becoming common to use several mutagenicity screening tests to detect potential carcinogenicity. Mutagenicity studies include tests with microorganisms (e.g., Ames assay), tests for genetic damage in cultured mammalian cells (e.g., unscheduled DNA repair synthesis, sister chromatid exchange, point mutations), and tests for *in vitro* transformation of rodent cell lines. Although the tests are extremely valuable for detecting chemicals requiring further study (i.e., animal bioassay and/or

epidemiology), they are not capable of detecting all potential carcinogens or indicating the relative potency of the carcinogens in humans.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

Studies on effects of pesticides and pesticide formulations are relatively standardized and include assays for acute eye irritation (OPPTS 870.2400), acute dermal irritation (OPPTS 870.2500), and skin sensitization (OPPTS 870.2600). The acute irritation studies typically involve rabbits. The test material is applied either to one eye of the animal or to an area of the skin (intact or abraded). In the eye irritation studies, the untreated eye of the animal typically serves as the control. In the dermal studies, an untreated area of the skin typically serves as a control. As summarized in Table 3-2, both eye and skin irritations studies are used to classify pesticides (corrosive to non-irritant) and these classifications reflect how the pesticide or pesticide formulations must be labeled.

Quantitative risk assessments for irritation are not derived in risk assessments conducted for the Forest Service or in risk assessments conducted by other organizations such as the U.S. EPA. Nonetheless, from a practical perspective, effects on the eyes or skin are overt effects that are frequently seen as a consequence of mishandling pesticides. These effects, however, can typically be avoided by proper industrial hygiene practices – e.g., wearing gloves or protective goggles to avoid or minimize exposure.

Eye and skin irritation studies may also be important in a risk assessment in evaluating the potential role of inert ingredients or adjuvants (Section 3.1.14). Along with acute oral toxicity studies (Section 3.1.4) and dermal toxicity studies (Section 3.1.12), studies on eye and skin irritation are often available on both the active ingredient as well as at least some commercial formulations. While comparisons between studies on the active ingredient and commercial formulations are not generally used quantitatively, substantial differences in the activity (e.g., irritant effects or other signs of toxicity) of the active ingredient and commercial formulation can be used to suggest that adjuvants or inert ingredients may or may not have a toxicologically significant effect.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Most of the occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of exposure. As discussed in Section 3.1.3.2, dermal absorption is estimated and compared to an estimated acceptable level of oral exposure based on subchronic or chronic toxicity studies. Nonetheless, any studies that are available on the dermal toxicity of the chemical are included in this section and an attempt is made to evaluate the estimates of dermal absorption rates that are derived in Section 3.1.3.2.

Most studies on the dermal toxicity of pesticides involve acute (single application) exposures and follow relatively standard protocols – e.g., acute dermal irritation assay given in OPPTS 870.2500. Occasionally, longer term subchronic studies such as the 28-day study given in OPPTS 870.2500 or the 90-day study given in OPPTS 870.3250 may be available but these are uncommon. As noted in previous sections, the dermal route may be used in some specialized

studies – e.g., reproductive effects – but these are not typically available for pesticides covered in Forest Service risk assessments. The general design and criteria for evaluating dermal studies are very similar to those for the corresponding oral studies and both range finding and limits tests may be conducted.

3.1.13. Inhalation Exposure

Inhalation toxicity studies can be as complex and varied as those involving oral exposure (Kennedy 1989; Klaassen et al. 1996; Wang et al. 1993). These may include standard acute toxicity studies observing gross signs of toxicity and gross tissue damage (OPPTS 870.1300), acute studies in which more detailed histopathologic examinations are made (OPPTS 870.1350), subchronic studies (OPPTS 870.3465), developmental studies (OPPTS 870.3600), and pharmacokinetic studies (OPPTS 870.8340). For most pesticides, particularly those covered in Forest Service risk assessments, inhalation is not a significant or substantial route of exposure (e.g., Ecobichon 1998; van Hemmen 1992).

The most commonly available study for pesticides reviewed in Forest Service risk assessments is the basic acute toxicity study (OPPTS 870.1300). Except for the route of exposure, 4-hours to a concentration of the chemical in air, the design of this study is generally similar to the acute oral toxicity study and range-finding studies as well as limit tests may be used. The limit test typically involves exposures to 2 mg/L.

3.1.14. Inerts and Adjuvants

Inert in this context refers to compounds which are intentionally added to a pesticide formulation but do not directly affect a pest species (EPA 1987). Inerts cover an extremely broad range of compounds including carriers, stabilizers, sticking agents, or other materials added to facilitate handling or application. However, these inerts may be toxic to humans or other non-target species. Thus, the U.S. EPA/OPP (1997) has recommended adopting the term “*other ingredients*” rather than inerts. The term *inerts*, however, continues to enjoy wide use and this term is still used in Forest Service risk assessment with the understanding that some inerts, which may be nontoxic to the target species, may present risks to both humans as well as other species.

The U.S. EPA is responsible for the regulation of inerts and adjuvants in pesticide formulations. As implemented, these regulations affect only pesticide labeling and testing requirements. As part of this regulatory activity, U.S. EPA classifies inerts into four lists based on the available toxicity information: toxic (List 1), potentially toxic (List 2), unclassifiable (List 3), and non-toxic (List 4). These lists as well as other updated information on pesticide inerts are maintained by the U.S. EPA at the following web site: <http://www.epa.gov/opprd001/inerts/>.

Any compound classified by U.S. EPA as toxic or potentially toxic must be identified on the product label if the compound is present at a level of 1% or greater in the formulation. All such compounds are considered explicitly in the risk assessment. If the compounds are not classified toxic, all information on the inert ingredients in pesticide formulations is considered proprietary under Section 10(a) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In that

case, the formulators of the pesticide need not and typically do not disclose the identity of the inert or adjuvant. The Northwest Coalition for Alternatives to Pesticides (NCAP) has obtained information on the identity of the inerts in several pesticide formulations from the U.S. EPA under the Freedom of Information Act and has listed this information on the NCAP web site (<http://www.pesticide.org/FOIA/clopyralid.html>). If information is available at this site, it is always included in the risk assessment. However, this site does not have information on the quantity of the inerts in the formulation. This information was not released by U.S. EPA and is treated as confidential.

Even if the identity of the inerts are known, the toxicity data available on inerts is often very limited. As discussed by Levine (1996), the testing requirements for inerts are less rigorous than the testing requirements for the pesticides (i.e., the active ingredients). Some standard sources are typically consulted for information on inerts, including the classifications on the U.S. EPA's inerts lists, discussed above. In addition, many inert ingredients are also approved food additives and the listing of approved food additives compiled by Clydesdale (1997) is also consulted. If an inert is on List 4 (nontoxic), concern for the inert is reduced. Similarly, if the inert is an approved food additive, concern is also reduced, particularly if the compound is classified as GRAS (generally recognized as safe). Some inerts that are potentially toxic have been reviewed and evaluated by other governmental groups. As discussed in Section 1.3.3, these reviews may be consulted to elaborate on the potential effects of the inert.

While concern may be diminished if the inert is a List 4 compound or if the inert is an approved food additive, the potential contribution of the inert is also assessed by comparing any available toxicity data on the active ingredient – i.e., the pesticide alone without any added adjuvants or inerts – to the toxicity of the formulated product. All pesticide formulations must identify the percent active ingredient in the formulation. As noted above, toxicity studies are often available on both the formulation and the active ingredient for acute oral exposures (Section 3.1.4) and acute dermal exposures (Section 3.1.12). Taking P as the proportion of the active ingredient in the formulation and LX as a toxicity value such as an LD_{50} or LC_{50} , the toxicity value for the formulation (LX_F) may be converted to the toxicity value in terms of the active ingredient (LX_A) by the simple formula:

$$LX_A = LX_F \times P \quad (\text{Eq. 3-10})$$

The toxicity value in terms of the active ingredient (LX_A) is then compared to experimental toxicity values on the active ingredient – i.e., LD_{50} or LC_{50} . For example, if the experimental LD_{50} for the active ingredient is substantially higher than the LX_A , this suggests that some components in the formulation may be contributing substantially to the toxicity of the formulation. Conversely, if the experimental LD_{50} for the active ingredient is substantially lower than the LX_A , this suggests that some components in the formulation may be antagonizing the toxicity of the active ingredient. While this sort of analysis is limited, it is often the only type of quantitative information that can be used to assess the toxicity of the formulated product.

In some rare cases – e.g., the Roundup formulation of glyphosate – very detailed information may be available on both the toxicity of the active ingredient, the toxicity of specific adjuvants, and the toxicity of the formulation. In such cases, very detailed chemical specific analyses can be and are conducted based on an assessment of toxicologic interactions (Section 3.1.16).

3.1.15. Impurities and Metabolites

In many respects, impurities and metabolites are much less difficult issues than inerts and adjuvants. Impurities often occur in pesticides. Virtually no chemical synthesis yields a totally pure product. Thus, technical grade pesticides undoubtedly contains some impurities. To some extent, concern for impurities in technical grade pesticides is reduced by the fact that the toxicity studies on pesticides are often conducted with the technical grade product. Thus, if toxic impurities are present in the technical grade product, they are likely to be encompassed by the available toxicity studies on the technical grade product.

An exception to this general rule involves carcinogens, most of which are presumed to act by non-threshold mechanisms. Because of the non-threshold assumption, any amount of a carcinogen in an otherwise non-carcinogenic mixture may pose a carcinogenic risk. An example of this is the occurrence of hexachlorobenzene in two herbicides used by the Forest Service, clopyralid and picloram. For these herbicides, the risk assessments each included a full dose-response assessment, exposure assessment, and risk characterization for the potential carcinogenic effects of hexachlorobenzene.

As discussed in Section 3.1.3.1, the assumption is generally made that studies on whole animals, such as those used to derive acceptable levels of exposure in humans, will encompass the toxicity of both the parent compound as well as any metabolites that formed *in vivo*. As also discussed in Section 3.1.3.1, however, this does not apply to toxic metabolites that are formed in the environment. In such a case, the toxicity of the metabolite as well as exposures to the metabolite may need to be quantitatively addressed in the risk assessment. Whether or not such steps are needed are discussed in this section of the risk assessment.

3.1.16. Toxicologic Interactions

Pesticides may sometimes be used in mixtures and the consequences of using mixtures may need to be assessed either from information on mechanism of action or direct toxicity data on the specific mixture. While Forest Service risk assessments do not directly deal with mixtures – i.e., risk assessment on pesticide mixtures are not explicitly considered in the exposure assessment, dose-response assessment, or risk characterization – any available information on toxicologic interactions is summarized in this section. As assessment of chemical interactions can be extremely complex and is data intensive (e.g., Mumtaz et al. 1994). Mechanistic bases for assuming that interactions might occur include an effect of one chemical on the pharmacokinetics of another chemical (e.g., absorption, metabolism, distribution, or excretion) or direct receptor interactions. For most pesticides, direct information on chemical interactions is very limited. When available, this information is discussed in this section of the risk assessment and may also be used in the assessment of connected actions in the risk characterization (Section 3.4.5).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

The exposure scenarios considered in a risk assessment involving pesticide exposure are determined by the application method and the chemical and toxicological properties of the compound. Depending on the properties of the chemical and the application method, the risk assessment may consider acute, subchronic, or chronic durations of oral, dermal, inhalation or combined exposure to the pesticide. Exposure scenarios are developed for workers, members of the general public, and various groups of nontarget species. For workers and the general public, two types of exposure scenarios are generally taken into consideration. They are *general exposure* and *accidental/incidental exposure*.

The term *general exposure* refers to human exposure resulting from the normal use of the chemical. For workers, general exposures involves the handling and application of the compound. These general exposure scenarios can be interpreted relatively easily and objectively. The exposure estimates are calculated from the amount of the chemical handled/day and the exposure rates for the worker group. Although each of the specific exposure assessments for workers involves degrees of uncertainty, the exposure estimates are objective in that they are based on empirical relationships of absorbed dose to pesticide use. For the general public, the general exposure scenarios are somewhat more arbitrary and may be less plausible. For each pesticide, at least three general exposures scenarios are considered, including walking through a contaminated area shortly after treatment, the consumption of ambient water from a contaminated watershed, and the consumption of contaminated vegetation. These three scenarios are consistently used because one of these three scenarios usually leads to the highest estimates of exposure. Additional scenarios discussed below may be considered for each of the individual compounds as warranted by the available data and the nature of the program activities.

Some, if not all, of these general exposure scenarios for the general public may seem implausible or at least extremely conservative. For example, in many cases, compounds are applied in relatively remote areas and so it is not likely that members of the general public would be exposed to plants shortly after treatment. Similarly, the estimates of longer-term consumption of contaminated water are based on estimated application rates (lbs a.i./acre) and monitoring studies that can be used to relate levels in ambient water to treatment rates in a watershed; however, in most pesticide applications, substantial proportions of a watershed are not likely to be treated. Finally, the exposure scenarios based on longer-term consumption of contaminated vegetation assume that an area of edible plants is inadvertently sprayed and that these plants are consumed by an individual over a 90-day period. While such inadvertent contamination might occur, it is extremely unlikely to happen as a result of directed applications (e.g., backpack applications). Even in the case of boom spray operations, the spray is directed at target vegetation and the possibility of inadvertent contamination of cultivated or edible vegetation would be low. In addition, for herbicides and other phytotoxic compounds, it is likely that the contaminated plants would show obvious signs of damage over a relatively short period of time and would therefore not be consumed.

All of the factors discussed above concerning general exposure scenarios for the general public are considered in the interpretation of the risk characterization (Section 3.4). Thus, the *typical* hazard to the general public may often be negligible because significant levels of exposure are not likely. For the general public, the general exposures may be regarded as *extreme* in that they are based on very conservative exposure assessments and/or very implausible events. Nonetheless, these general exposure assessments are included because the risk assessment is intended to be extremely conservative with respect to potential effects on the general public, and to provide estimates regarding the likelihood and nature of effects after human exposure to pesticides.

Accidental/incidental exposure scenarios describe specific examples of gross over-exposure associated with mischance or mishandling of a chemical. All of these exposure scenarios are arbitrary in that the nature and duration of the exposure is fixed. For example, the worker exposure scenario involving immersion of the hands is based on a 1-minute period of exposure but could just as easily be based on an exposure period of 5 seconds or 5 minutes. Similarly, the consequences of wearing contaminated gloves could be evaluated at 4 hours rather than at 1 hour. These scenarios are intended to provide an indication of relative hazard among different pesticides and different events in a manner that facilitates conversion or extrapolation to other exposure conditions.

Like the general exposure scenarios, the accidental exposures for the general public may be regarded as more extreme than those for workers. Three scenarios are included in each exposure assessment. They include direct spray, the consumption of contaminated water shortly after a spill, and the consumption of contaminated vegetation shortly after treatment. The direct spray scenario is clearly extreme. It assumes that a naked child is sprayed directly with a pesticide as it is being applied and that no steps are taken to remove the pesticide from the child for 1 hour. There are no reports of such incidents in the literature, and the likelihood of such an incident occurring appears to be remote. Nonetheless, this scenario and others like it are useful not only as a uniform comparison among pesticides but also as a simplifying step in the risk assessment. If the '*naked child*' scenario indicates no basis for concern, other dermal spray scenarios will not suggest a potential hazard and need not be explored. If there is a potential hazard, other more plausible exposure scenarios may need to be considered. The other two accidental scenarios are similarly motivated as uniform comparisons among chemicals as well as a means of evaluating the need to explore additional exposure scenarios.

In all cases, the level of exposure is directly proportional to the exposure parameters. The exposure associated with wearing gloves for 4 hours is 4 times the exposure associated with wearing contaminated gloves for 1 hour. Similarly, the general exposure scenarios for workers are based on an 8-hour work day. If a 4-hour application period were used, the hazard indices would be reduced by a factor of two. As another example, general exposure scenarios for both workers and the general public are linearly related to the application rate. Consequently, if the application rate were to double or vary by some other factor, the estimated exposure would double or vary by the same factor. Thus, the specific exposure parameters used in the risk

assessment are selected to allow for relatively simple extrapolation to greater or lesser degrees of exposure.

Additional variability is taken into consideration by estimating exposure doses or absorbed doses for individuals of different age groups (i.e., adults, young children, toddlers, and infants). Children may behave in ways that increase the exposure to applied pesticides (e.g., long periods of outdoor play, pica, or imprudent consumption of contaminated media or materials). In addition, anatomical and physiological factors such as body surface area, breathing rates, and consumption rates for food and water, are not linearly related to body weight and age. Consequently, the models used to estimate the exposure dose (e.g., mg/kg body weight/day) based on chemical concentrations in environmental media (e.g., ppm in air, water, or food) indicate that children, compared with individuals of different age groups, are generally exposed to the highest doses of chemicals for a given environmental concentration.

3.2.2. Workers

The potential exposures of and health effects in pesticide applicators is a major focus in such USDA risk assessments. The concern for worker exposure is motivated by obvious ethical considerations as well as the understanding that pesticide applicators are likely to be the individuals who are most exposed to the pesticide during the application process.

Two general types of methods can be considered for worker exposure modeling, deposition-based and absorption-based. The U.S. EPA's Office of Pesticide Programs employs a deposition-based approach using data from the Pesticide Handler's Exposure Database (PHED Task Force 1995). In this type of model, the exposure dose is estimated from air concentrations and skin deposition monitoring data. Using these estimates, the absorbed dose can be calculated if estimates are available on absorption rates for inhalation and dermal exposure.

The USDA Forest Service has generally used absorption-based models in which the amount of chemical absorbed is estimated from the amount of chemical handled (e.g., USDA/FS 1989a,b,c). Absorption-based models rather than deposition-based models have been used by the Forest Service because of two common observations from field studies. First, as discussed in the review by van Hemmen (1992), most studies that attempt to differentiate occupational exposure by route of exposure indicate that dermal exposure is much greater than inhalation exposure for pesticide workers. Second, most studies of pesticide exposure that monitored both dermal deposition and chemical absorption or some other method of biomonitoring noted a very poor correlation between the two values (e.g., Cowell et al. 1991; Franklin et al. 1981; Lavy et al. 1982). In USDA Forest Service exposure assessments for workers, the primary goal is to estimate absorbed dose so that the absorbed dose estimate can be compared with available information on the dose-response relationships for the chemical of concern. Thus, if dermal deposition does not correlate well with absorbed dose and if the inhalation route is not a substantial factor in worker exposure, the absorption-based approach may have some advantages when compared to the deposition-based approach.

Both the deposition method used by the U.S. EPA (PHED Task Force 1995) as well as the general estimates of worker exposure rates currently used in risk assessments for the Forest Service may be viewed as relatively crude approximations. As better data become available and methods to use this data are refined, additional methods may be employed. For example, Durkin et al. (2004) have developed a physiologically based pharmacokinetic model for 2,4-D, and have demonstrated that the model can be used to fit the variability in worker exposure to 2,4-D from the study by Lavy et al. (1982). This analysis involved a combination of both deposition data from PHED data base and measurements of dermal absorption rates. Using this approach, the central estimates of risk to workers were virtually identical to the estimates obtained from the general approach detailed below. As kinetic models are developed for other pesticides, they may be used in place of either the deposition or simple absorption rate estimates that are currently used.

3.2.2.1. General Exposures – Initially, SERA's risk assessment for the Forest Service adjusted the exposure rate by the estimated dermal absorption rate, typically using 2,4-D as a surrogate chemical when compound specific data were not available. Subsequently, SERA (1998) conducted a detailed review and re-evaluation of the available worker exposure studies that can be used to relate absorbed dose to the amount of chemical handled per day. This review noted that there was no empirical support for a dermal absorption rate correction. Two factors appear to be involved in this unexpected lack of association:

algorithms for estimating dermal absorption rates have large margins of error

and

actual levels of worker exposure are likely to be far more dependent on individual work practices or other unidentified factors than on differences in dermal absorption rates.

Thus, in the absence of data to suggest an alternative approach, no corrections for differences in dermal absorption rate coefficients or other indices of dermal absorption seem to be appropriate for adjusting occupational exposure rates.

Although pesticide application involves many different job activities, exposure rates can be defined for three categories: directed foliar applications including cut surface, streamline, and direct sprays involving the use of backpacks or similar devices; broadcast hydraulic spray applications; and broadcast aerial applications. While these may be viewed as crude groupings, the variability in the available data do not seem to justify further segmenting the job classifications - e.g., hack-and-squirt, injection bar. All of the details of the worker exposure calculations are summarized in standard worksheets that are typically designated as C01a (directed ground), C01b (broadcast ground), and C01c (aerial). The specific exposure rates used for each groups are summarized in Table 3-3 and discussed below.

Directed Foliar Applications. Based on the data reviewed by SERA (1998), the mean (with 95% confidence interval) of the exposure rates for all ground workers involved in directed foliar backpack applications is about 5.3×10^{-3} (2.4×10^{-4} to 9.7×10^{-3}) mg/kg/lb a.i. handled. The mean and the confidence interval are based on a log normal distribution (mean=0.005297, SD=0.00232, p=0.46 using Kolmogorov-Smirnov test). These estimates are based on the mean data on glyphosate (Jauhiainen et al. 1991), triclopyr BEE (Middendorf 1992), picloram (Libich et al. 1984), and 2,4-D (Libich et al. 1984), but the estimates exclude the backpack applicators from Lavy et al. (1987) because of the atypical and very heavy dermal contact.

An alternate analysis for backpack workers can be based on individual data points rather than means for each of the four chemicals. However, as detailed in the appendix, the estimate for glyphosate from the Jauhiainen et al. (1991) study involved making several assumptions concerning urinary levels that are not as well supported as the measured values from the studies reported by Libich et al. (1984) and Middendorf (1992) - although the resulting estimates of absorbed dose rate from the Jauhiainen study are very consistent with those from the other two studies. For this reason, no data from Jauhiainen et al. (1991) were included in the analysis based on individual data. Two other points were censored from the analysis, workers G and H from the study by Middendorf (1992). As detailed by Middendorf (1992), neither of these two workers wore gloves and both had levels of exposure that were atypically high. Thus, data on a total of 16 workers can be used in the analysis of the individual worker exposure rates, 14 from the Middendorf (1992) study and two from the Libich et al. (1984) study. These individual data also fit a log normal distribution (p=0.78 using Kolmogorov-Smirnov test. The mean (with 95% confidence interval) of the absorbed rate for these 16 workers is about 3.2×10^{-3} (3.4×10^{-4} to 1.0×10^{-2}) mg/kg/lb a.i. handled and the standard deviation of the estimate is 0.00457.

Although the estimates based on the averages for four chemicals are not substantially different from the analyses based on the individual data points for three chemicals, the analysis based on individual workers is used for estimated exposures from directed foliar application. The available data on other application methods (i.e., hack-and-squirt and injection bar) suggest exposure rates that may be less than those of backpack workers by about a factor of 2. This difference is not substantial or statistically significant. Since the estimate of the magnitude of the difference is based on only two studies and two chemicals, the better documented rate for backpack applicators is recommended for these other types of manual ground applications. Thus, rounding to one significant digit, the recommended values for all forms of directed backpack applications are 0.003 (0.0003 to 0.01) mg/kg/lb a.i. handled.

Broadcast Ground Applications. Estimates of worker exposures from ground broadcast applications are based on two published studies of occupational exposure rates involving hydraulic spray applications, Libich et al. (1984) and Nash et al. (1982). The analysis of both of these studies is detailed in SERA (1998). The Libich et al. (1984) study involved mixtures of 2,4-D, dichlorprop, and picloram. Although this study is very useful for assessing relative rates of exposure, the amounts handled by the workers are not specified, so this study is not suitable for calculating absolute occupational exposure rates. The study by Nash et al. (1982) provides all

of the necessary information on 21 of 26 workers (i.e., Table V in Nash et al. 1982), including the amount handled, the duration of application, and the total urinary elimination of 2,4-D over a 6-day post-application period. For the remaining five workers, the amount handled was not recorded for four of the workers and 2,4-D was not detected in the urine of one of the remaining workers. In recent Forest Service risk assessments, this study was used to support occupational exposure rates of 9.6×10^{-5} mg/kg bw/lb a.i., with lower and upper 95% confidence limits of 4.9×10^{-6} and 1.9×10^{-3} mg/kg bw/lb a.i.

The re-analysis of these data was conducted excluding the four workers on which the data are not complete and using a trimmed mean (Gilbert 1987) to account for the one worker in which no 2,4-D was detected - i.e., omitting the data for the worker with no detectable 2,4-D in the urine as well as the data for the worker with the highest reported level of 2,4-D in the urine. These individual occupational exposure rates fit a log normal distribution [$p=0.78$ using the Kolmogorov-Smirnov test (Manugistics 1997) with an n of 20] with a mean of 2.4×10^{-4} mg/kg bw/lb a.i., a standard deviation of 5.48×10^{-4} and 95% confidence limits of 1.1×10^{-5} to 9.00×10^{-4} mg/kg bw/lb a.i. Again rounding to one significant figure, the recommended exposure rates for broadcast aerial applications are 0.0002 (0.00001 to 0.0009) mg/kg bw/lb a.i.

Broadcast Aerial Applications. As discussed in SERA (1998), data on aerial crews applying 2,4-D (Lavy et al. 1982; Nash et al. 1982) as well as data on mixers using cypermethrin from the study by Chester et al. (1987) are useful for estimating exposure rates pertinent to aerial applications. The lack of an apparent relationship between estimated rates for dermal absorption and occupational exposure, as discussed above, suggests that it is appropriate to combine the data on cypermethrin with the data on 2,4-D.

Like data sets for ground applications, the individual occupational exposure rates fit a log normal distribution [$p=0.91$ using the Kolmogorov-Smirnov test (Manugistics 1997)]. There is at least a marginally significant ($p=0.032$) correlation between the log of the absorbed dose and the log of the amount handled, although the correlation coefficient between these two variables is low ($r^2=0.19$).

Using a log normal distribution, the mean of the rates is 3.08×10^{-5} mg/kg bw/lb a.i., with lower and upper 95% confidence limits of 1.08×10^{-6} to 1.16×10^{-4} mg/kg bw/lb a.i. These rates are applied to both pilots as well as mixer/loaders. Given the relatively minor and statistically insignificant differences in occupational exposure rates between pilots and mixer/loaders, separate exposure rates for these two groups are not justified. There are insufficient data on other job categories in aerial applications to support the derivation of additional occupational exposure rates. Thus, for both pilots and mixer-loaders, the recommended exposure rates are 0.00003 (0.000001 to 0.0001) mg/kg bw per lb a.i. applied.

Studies involving occupational exposure during aerial applications of 2,4,5-T suggest that flaggers—ground personnel who mark the area to be sprayed—are likely to have lower exposure rates than mixer/loaders. In a study by Lavy et al. (1980) involving exposure of aerial crews to

2,4,5-T, the amounts of 2,4,5-T handled are not specified and exposures are characterized in units of mg 2,4,5-T/kg bw based on urinalysis. Although this study cannot be used to calculate occupational exposure rates, information is reported on mixers and flagmen in two crews. Since each crew, by definition, handled the same amount of material per day, this information can be used to estimate relative differences in exposure. For mixers in the two crews, the daily excretion of 2,4,5-T was 0.065 mg/kg bw and 0.096 mg/kg bw. For the two flagmen in the first crew, the daily excretion was 0.002 mg/kg and 0.001 mg/kg. In the second crew, the daily excretion for both flagmen was 0.001 mg/kg. Thus, the average excretion rate for the mixers [0.081 mg/kg] was about 65-fold greater than the average rate for flagmen [0.00125 mg/kg]. Conversely, the exposure estimate for flagmen was about 1.5% of that estimated for mixers.

Similarly, Newton and Norris (1981) briefly reported about exposure for mixer-loaders and flaggers in a helicopter crew spraying 2,4,5-T. Absorbed doses for mixer-loaders ranged from 0.016 to 0.063 mg/kg, in the range of absorbed doses reported by other investigators. A flagger, wearing a hat and long-sleeved shirt, absorbed 0.005 mg/kg. The report does not provide information regarding the amount of the chemical handled or sprayed, or the duration of activities associated with the exposures. In a review, Lavy and Mattice (1985) provide similar information from an unpublished study: absorbed doses of 0.001 mg/kg for flagmen and 0.062 mg/kg for mixers. Thus, it seems reasonable to suggest that occupational exposure rates for flaggers, barring accidental direct sprays, will be about 10-100 lower than the rates for pilots or mixer/loaders.

3.2.2.2. Accidental Exposures – Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route for herbicide applicators (Ecobichon 1998; van Hemmen 1992). Typical multi-route exposures are encompassed by the methods used in Section 3.2.2.1 on general exposures. Accidental exposures, on the other hand, are most likely to involve splashing a solution of herbicide into the eyes or to involve various dermal exposure scenarios.

For most risk assessments, two quantitative exposure scenarios are developed for each of two types of dermal exposure: spilling a chemical onto the surface the skin and wearing clothing that is contaminated with the pesticide. For body types of exposures, the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of an herbicide for any period of time. On the other hand, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant.

For both scenarios (the hand immersion and the contaminated glove), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA/ORD (1992), Fick's first law is used to estimate dermal exposure. If an experimental dermal permeability coefficient (K_p) for the compound is not available, the K_p is estimated using the algorithm from U.S. EPA/ORD (1992). The basic algorithm is given in Equation 3-1. Confidence limits for the K_p are calculated based on the data set used by U.S. EPA/ORD (1992) and these calculations are always detailed in a worksheet. In most Forest Service risk assessments, these details for calculating the confidence intervals are provided in Worksheet A07b and the specific confidence intervals for the chemical are given in Worksheet B04.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill onto the lower legs as well as a spill onto the hands. In these scenarios, it is assumed that a solution of the chemical is spilled onto a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the concentration of the chemical in the liquid), the first-order absorption rate, and the duration of exposure. For both scenarios, it is assumed that the contaminated skin is effectively cleaned after 1 hour.

If an experimental first-order dermal absorption rate is available (Section 3.1.3.2), this value is generally used directly. If not, the first-order dermal absorption rates is calculated based on Equation 3-3. As with the exposure assessments based on Fick's first law, this product (mg of absorbed dose) is divided by body weight (kg) to yield an estimated dose in units of mg chemical/kg body weight. The specific equations used in these exposure assessments are typically specified in Worksheet B03.

3.2.3. General Public

3.2.3.1. General Considerations – Depending on the use and application method of the pesticide under review, members of the general public may or may not likely be exposed to the pesticide. For example, exposures are likely and virtually certain for chemicals that are used in broadcast aerial spray in inhabited areas. Such chemicals could be insecticides that are used to control insect pests. On the other hand, exposures are less likely for some herbicides that are used only in spot applications. Nonetheless, any number of exposure scenarios can be constructed for the general public, depending on various assumptions regarding application rates, dispersion, canopy interception, and human activity. Risk assessments prepared for the Forest Service typically include several sets of scenarios that are intended to characterize exposure in a consistent manner that allows for comparisons among different pesticides that the Forest Service might consider.

The two general types of exposure scenarios developed for the general public include acute exposure and longer-term (chronic exposure). Most of the acute exposure scenarios are accidental and assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, as well as the consumption of contaminated fruit, water, and fish. Most

of these scenarios should be regarded as extreme, some to the point of limited plausibility. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, and fish but are based on estimated levels of exposure for longer periods after application.

As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany the risk assessment. These worksheets are described in Appendix 3. This section focuses on a qualitative description of the rationale for and the types of data used in each type of exposure scenario.

3.2.3.2. *Direct Spray* – Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). The scenario involves an individual sprayed with a solution containing the compound. The assumption is made that an amount of the compound remains on the skin and is absorbed by first-order kinetics. For these exposure scenarios, it is assumed that during a ground application, a naked child is sprayed directly with the compound. These scenarios also assume that the child is completely covered (that is, 100% of the surface area of the body is exposed). These exposure scenarios are likely to represent upper limits of plausible exposure. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs. For each of these scenarios, some assumptions are made regarding the surface area of the skin and body weight. These assumptions are based on standard values developed by the U.S. EPA (U.S. EPA 1989a,b) which are detailed in the worksheets.

3.2.3.3. *Dermal Exposure from Contaminated Vegetation* – In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate, and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. For most pesticides, no data are available on dermal transfer rates and the estimation methods of Durkin et al. (1995). These are detailed in the worksheets, typically in Worksheet D02. The exposure scenario assumes a contact period of one hour and assumes that the chemical is not effectively removed by washing for 24 hours. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

3.2.3.4. *Contaminated Water* – Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, or from unintentional contamination from spray drift. For most risk assessments, the three types of estimates made for the concentration of the pesticide in ambient water: accidental acute exposure from an accidental spill, acute peak exposures from normal applications, and longer-term exposure in ambient water that could be associated with normal applications. In modeling normal applications, the size of the treated area as well as the characteristics of the body of water are important parameters. Typical assumptions involve the application of the compound to a 10 acre block that is adjacent to and drains into a

small stream or pond. Other assumptions of different treatment areas and/or different bodies of water may be made depending on the specific uses and application methods for the pesticide.

3.2.3.4.1. ACUTE EXPOSURE – Two exposure scenarios are presented for the acute consumption of contaminated water: an accidental spill into a small pond (0.25 acres in surface area and 1 meter deep) and the contamination of a small stream by runoff or percolation.

The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill into a small pond. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of the chemical is considered. This scenario is dominated by arbitrary variability and the specific assumptions used will generally overestimate exposure. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. The other acute exposure scenario for the consumption of contaminated water involves runoff into a small stream or pond.

If relevant monitoring data are available, these data are discussed in this section and are used to evaluate the modeled estimates. In this context, relevant monitoring data include any studies that report concentrations in a stream or pond at known intervals after a defined application of the compound. To the extent that meteorologic or other site-specific data are available, these data may be used to more fully evaluate the modeled estimates (discussed below). If the modeling data and monitoring data are not concordant, the exposure estimates used in the risk assessment may be based on values (modeled or monitored) that lead to the highest estimates of exposure – i.e., the most conservative approach.

While monitoring data provide practical and documented instances of water contamination, monitoring studies may not encompass a broad range of conditions which may occur during program applications – e.g., extremely heavy rainfall – or they may reflect atypical applications that do not reflect program practices. Consequently, for this component of the exposure assessment, modeled estimates are made based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems).

GLEAMS is a root zone model that can be used to examine the fate of chemicals in various types of soils under different meteorological and hydrogeological conditions (Knisel and Davis 2000). As with many environmental fate and transport models, the input and output files for GLEAMS can be complex. The general application of the GLEAMS model and the use of the output from this model to estimate concentrations in ambient water are detailed in SERA (2003b).

As noted above, the application site is typically assumed to consist of a 10 acre square area that drained directly into a small pond or stream. The chemical specific values as well as the details of the pond and stream scenarios used in the GLEAMS modeling are always explicitly summarized in a table which indicates the input parameters and bases for the input parameters.

The GLEAMS model yields estimates of runoff, sediment loss, and percolation that are used to estimate concentrations in the stream adjacent to a treated plot. The specific methods that are used are detailed in Appendix 1. The results of the GLEAMS modeling for the small stream and the corresponding values for the small pond are expressed as both average and maximum water contamination rates (WCR) - i.e., the concentration of the compound in water in units of mg/L normalized for an application rate of 1 lb/acre.

The GLEAMS scenarios do not specifically consider the effects of accidental direct spray or spray drift. For example, the stream modeled using GLEAMS is about 6 feet wide and it is assumed that the herbicide is applied along a 660 foot length of the stream with a flow rate of 4,420,000 L/day. At an application rate of 1 lb/acre, accidental direct spray onto the surface of the stream would deposit about 41,252,800 μg [$1 \text{ lb/acre} = 112,100 \mu\text{g/m}^2$, $6' \times 660' = 3960 \text{ ft}^2 = 368 \text{ m}^2$, $112,100 \mu\text{g/m}^2 \times 368 \text{ m}^2 = 41,252,800 \mu\text{g}$]. This would result in a downstream concentration of about 10 $\mu\text{g/L}$ [$41,252,800 \mu\text{g/day} \div 4,420,000 \text{ L/day}$]. These types of estimates from drift will be made as appropriate in each risk assessment. If the modeled estimates from GLEAMS or the available monitoring data lead to estimates that are substantially lower than the drift calculations, the estimates from these drift calculations may be used directly in setting the upper bounds of the range of exposures that are used in the risk assessment.

3.2.3.4.2. LONG-TERM EXPOSURE – The scenario for chronic exposure from contaminated water assumes that an adult (70 kg male) consumes contaminated ambient water from a contaminated pond for a lifetime. The estimated concentrations in pond water are based on the modeled estimates from GLEAMS, discussed in the previous section. As with the estimates of peak exposure, the longer-term exposures are expressed as water contamination rates (WCR) - i.e., the concentration of the compound in water in units of mg/L normalized for an application rate of 1 lb/acre. As with the estimates of acute or peak concentrations, the longer-term estimates of concentrations may be based directly on modeled data or monitoring data, depending on the quality of the monitoring data that are available, confidence in the modeled estimates, and an assessment of which types of data would lead to the most conservative plausible estimates of exposure.

3.2.3.5. Oral Exposure from Contaminated Fish – Many chemicals may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. Bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the bioconcentration factor (BCF) is 5 L/kg [$5 \text{ mg/kg} \div 1 \text{ mg/L}$]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. Details regarding the relationship of bioconcentration factor to standard pharmacokinetic principles are provided in Calabrese and Baldwin (1993).

The potential for accumulation of a pesticide or other chemical in fish is typically studied in bluegill sunfish, trout, minnows, or occasionally carp. The U.S. EPA (2005) has a general

protocol for this type of study, OPPTS 850.1730. The fish are typically exposed to at least two concentrations of the radiolabeled (e.g., ^{14}C) chemical in water for periods of 28 to 60 days. At steady state, the bioconcentration factor (BCFs) is calculated as the ratio of the concentration in the fish (C_f) to that in the water (C_w). For most water soluble chemicals, steady state will typically be reached during the 28 to 60 day exposure period. If this is not the case, kinetic analyses similar to those discussed in Section 3.1.3.3 may be used to calculate the BCF.

Typically, two sets of BCF values are given in each risk assessment, one for acute (24 hour) exposures and the other for longer-term (steady-state) exposures. Again, for many water soluble chemicals, these two estimates will not differ substantially. For highly lipophilic compounds – i.e., compounds that will partition into fat and other lipids – the values will differ substantially. In addition, separate BCF values are typically given for whole fish and the edible portion of fish.

For both the acute and longer-term exposure scenarios involving the consumption of contaminated fish, the concentrations of the chemical in water that were used to estimate concentrations in fish are identical to the concentrations used in the contaminated water scenarios (see Section 3.2.3.4). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or about one-quarter acre. No dissipation or degradation is considered. Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and subsistence populations (U.S. EPA/ORD 1996a), separate exposure estimates are made for these two groups. The chronic exposure scenario is constructed in a similar way, except that estimates of concentrations of the chemical in ambient water are based on the longer-term estimates discussed in Section 3.2.3.4.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although Forest Service applications of pesticides will not generally involve the intentional treatment of food crops, incidental exposure to vegetation that may be consumed by members of the general public is plausible during aerial or broadcast applications. Any number of scenarios could be developed involving either accidental spraying of crops or the spraying of edible wild vegetation, such as berries. The exposure scenarios developed in most risk assessments include one scenario for acute exposure and one or two scenarios for longer-term exposure (depending on the number of applications per year). In both acute and longer-term scenarios, the concentration of the chemical on contaminated vegetation is typically estimated using the empirical relationships between application rate and concentration on vegetation developed by Fletcher et al. (1994), which is in turn based on a re-analysis of data from Hoerger and Kenaga (1972).

For the acute exposure scenario involving only a single application, the estimated residue level is taken as the product of the application rate and the residue rate for contaminated fruit. For multiple applications, the peak concentration on fruit or other vegetation will occur immediately after the last application. This concentration can be calculated based on the initial concentration after the first application (C_0), the number of applications (n), and the first-order decay

coefficient (k), which can be calculated from the halftime (t_{50}) [$k=\ln(2)\div t_{50}$]. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after the first application (C_t), can be calculated as:

$$C_t = C_0 \times e^{-kt} \quad (\text{Eq. 3-11})$$

Using the plateau principle (Section 3.1.3.3), defining t^* as the interval between applications and defining $e^{-k t^*}$ as p to simplify notation, the concentration immediately after the n^{th} application (C_n) can be calculated as:

$$C_n = C_0 \times (1 - p^n) \div (1 - p) \quad (\text{Eq. 3-12})$$

This algorithm is used to calculate the maximum concentration on vegetation after multiple applications at the specified interval.

For the longer-term exposure scenarios, a duration of 90 days is typically used. Although the duration of exposure of 90 days is somewhat arbitrary, this duration is intended to represent the consumption of contaminated fruit that might be available over one season. Longer durations could be used for certain kinds of vegetation but would lower the estimated dose (i.e., would reduce the estimate of risk).

Estimates of halftimes on vegetation can come from either field studies or greenhouse studies and may be highly variable. Substantial variability is not uncommon in field measurements of halftimes of vegetation, which are substantially impacted by site and situational differences such as rainfall, temperature, wind velocity, and vegetation type.

For the longer-term exposure scenarios, the time-weighted average concentration on vegetation or fruit is calculated from the equation for first-order dissipation. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after spray, C_t , can be calculated based on the initial concentration, C_0 , as:

$$C_t = C_0 \times e^{-kt} \quad (\text{Eq. 3-13})$$

where k is the first-order decay coefficient which can be calculated from the halftime (t_{50}) [$k=\ln(2)\div t_{50}$]. For a single application, the time-weighted average concentration (C_{TWA}) over time t can be calculated as the integral of C_t (De Sapio 1976, p. p. 97 ff) divided by the duration (t):

$$C_{\text{TWA}} = C_0 (1 - e^{-k t}) \div (k t). \quad (\text{Eq. 3-14})$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single application.

For pesticides which may be applied twice per year, the expression of the time-weighted average concentration is somewhat more complicated. Defining $\exp(x)$ as e^x , where x is any number, the time-weighted average concentration over a period from the day of application to time t_2 with a second application occurring on day t_1 (where t_1 is less than t_2) is:

$$C_{TWA} = (C_0 (1-\exp(-kt_1)) + [\{C_0 + C_0 \exp(-kt_1)\} \times \{1-\exp(-k [t_2 - t_1])\}]) \div (k t_2) \quad (\text{Eq. 3-15})$$

This equation is used to estimate the time-weighted average concentration on vegetation after two applications.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The purpose of the dose-response assessment is to describe the degree or severity of risk as a function of dose. Most dose-response assessments used in Forest Service risk assessments are in the form of RfDs or Reference Doses. These are point estimates (single numbers rather than ranges) of doses that are not believed to be associated with any adverse effect and that are not directly related to a dose-response model. The practice of relying on point estimates in regulatory toxicology is grounded in the history of this discipline (Dourson and Stara 1983). From its inception, the focus of regulatory toxicology has been the development of criteria (i.e., levels of exposure) that are defined as *safe*. Consequently, the methods used in regulatory toxicology are conservative. Consistent with the recommendation of NRC (1983) that various groups within the federal government adopt common risk assessment methodologies, standard dose-response assessments are generally based on reference values, like RfDs, derived by other government agencies, most commonly the U.S. EPA. This approach avoids a duplication of effort, capitalizes on the expertise of other organizations, and decreases the size, complexity, and cost of risk assessments.

In classical toxicology, dose-response assessments are usually expressed as linear or non-linear equations such as probit analysis and the multistage model, respectively. Using these methods, the prevalence or magnitude of a response can be estimated for any dose level. This kind of approach is being used more in risk assessments as a method of more fully using the available data, as well as a method of incorporating estimates of variability. The most commonly used method involves the calculation of benchmark doses which may be used as a surrogate for NOAEL values. This sort of method is also used in many cancer risk assessments. For example, U.S. EPA cancer risk assessments usually employ a form of the multistage model or some other linear dose-response relationship that provide measures of variability or error.

In cases for which these standard approaches yield evidence of potential risk, qualitative or quantitative approaches may be taken to characterize the potential effects that might be seen. Qualitative methods simply involve attempting to describe the types and severity of responses that might be seen at different levels of exposure. Statistical methods such as categorical regression may also be used to characterize the likelihood and severity of the risk. This technique defines a relationship between responses that can be categorized according to exposure dose and duration (factors that may influence the response), and estimates the probability that a group of animals subjected to a given exposure will be classified into a particular category (Dourson et al. 1997, Durkin et al. 1992, Guth et al. 1997).

3.3.2. Chronic RfD

Chronic RfDs are used to characterize risks associated with chronic or longer-term exposures. These values are typically based on NOAEL values from chronic or subchronic toxicity studies (Section 3.1.5) or on multigeneration reproductive studies (Section 3.1.9.2). The selection of the specific NOAEL value depends on which endpoint appears to be the most sensitive – i.e., the NOAEL associated with the LOAEL; the lowest observed adverse effect level is identified. This

is viewed as the *critical effect* and may be any systemic toxic effect or any effect on reproduction. If there is an NOAEL associated with the LOAEL (an NOAEL from the same study), then this NOAEL is used as the basis for the RfD. If no NOAEL is available, the LOAEL may be used with an additional uncertainty factor as discussed below.

Chronic RfD values are intended to estimate dose levels associated with a negligible or at least defined level of risk over a lifetime of exposure. *RfD* is a term used by U.S. EPA to designate a *Reference Dose* for use in risk characterization and most RfD values used in Forest Service risk assessments are those that are derived by U.S. EPA. Occasionally, RfD values are derived by other organizations or RfD values are derived in the risk assessment itself. These situations arise primarily in cases in which the U.S. EPA has not derived an RfD. To avoid confusion, any RfD value that is not derived by the U.S. EPA is generally referred to as a *surrogate RfD* in a Forest Service risk assessment. Specific examples of these types of dose-response assessments, including RfDs and similar values, conducted by various governmental organizations, are provided in Table 3-4.

The risk assessment may use acute or chronic RfDs. The definitions of acute, subchronic, and chronic exposure are vague, and to some extent, chemical specific, as discussed in Section 3.1.3. If 1-day, 10-day, or longer-term health advisories (HAs) are available (see Table 3-4), these values may be used to derive acute or subchronic RfDs. Acute RfD values are conceptually similar to chronic RfD values and the major difference is in the type of study used to derive an acute RfD. This is discussed further in Section 3.3.3. RfD values are used primarily for non-carcinogenic chemicals. Quantitative dose-response assessments for carcinogenicity are discussed in Section 3.3.7.

Non-carcinogenic effects are assumed to have population thresholds (i.e., levels below which no adverse effects are expected for a given exposure route and duration). RfDs for non-carcinogenic effects are intended to be estimates of exposure levels at or below the threshold level. The basic equation for deriving an RfD is very simple and can be expressed as:

$$\text{RfD} = \text{TV}/\text{UF} \quad (\text{Eq. 3-16})$$

where TV is an experimental toxicity value such as an NOAEL or LOAEL and UF is the uncertainty factor. Although the computations are simple, the toxicological judgments involved in deriving a reference value may be complex. To derive the RfD, the experimental threshold or NOAEL is divided by the product of a series of uncertainty factors intended to account for differences between the experimental exposure and the conditions for which the reference value is derived. The uncertainty factors used by the U.S. EPA and ATSDR are presented in Table 3-5.

In assessing dose-severity relationships, the emphasis is on distinguishing the range of doses over which adverse effects were observed from the range of doses over which no adverse effects were observed. As discussed in Section 3.1.5.3, the classification of an effect as adverse or adaptive can be highly judgmental.

For oral RfDs, units of dose usually are expressed as mg/kg/day. For inhalation exposures, the term RfC (Reference Concentration) is typically used and the value is expressed as mg/m³. Data on certain species may be censored from the analysis because they are atypical and do not serve as good animal models for effects in humans. An attempt is then made to determine the most sensitive toxicological endpoint. Usually, this is accomplished by identifying a toxicologically relevant series of effects that increase in severity as dose increases.

Generally, the risk assessment will use U.S. EPA RfDs as the toxicity value for risk characterization. U.S. EPA RfDs generally provide a level of analysis, review, and resources that far exceed those that are or can be conducted in support of most Forest Service risk assessments. In addition, it is desirable for different agencies and organizations within the federal government to use concordant risk assessment values.

Nonetheless, there are cases in which different RfDs for the same chemical are derived within the U.S. EPA and other cases in which the nature of the available data suggest the need to use alternative values to capture endpoint-specific toxicities, dose-duration relationships, or dose-severity relationships as adequately as possible. Lastly, there may be cases where new data are available – i.e., data not considered by or available to the U.S. EPA when the RfD was derived. Sometimes, the alternative values are less conservative; at other times, the alternative values are more conservative. In either case, the purpose of deviating from the U.S. EPA RfDs is to characterize risk as clearly and thoroughly as possible.

Typically, inhalation exposures to pesticides used by the Forest Service are marginal and no explicit dose-response assessment is provided for inhalation exposures. When inhalation exposures are explicitly considered, RfD values derived by U.S. EPA are used if available. As an alternative, Threshold Limit Values (TLVs) derived by American Conference of Governmental Industrial Hygienists may serve as the basis for inhalation RfDs. These may be adopted without modification as inhalation RfDs for occupational exposure. For exposure scenarios involving the general public, inhalation RfCs may be adopted without modification as inhalation RfDs for chronic exposure. When RfCs are not available, the TLV may be modified to account for the duration of daily exposure and sensitive subgroups within the general population. TLVs are designed to protect workers in occupational exposure settings during the work day (8 hours/day). Inhalation RfDs for the general public must be protective for the full 24-hour day. Consequently, the TLV will be reduced by one third (8 hours/24 hours) when applied to the general public. This adjustment is made with the assumption that exposures are equitoxic as long as the product of concentration and duration is constant (e.g., $c_1 d_1 = c_2 d_2$). This is an expression of Haber's law (Kennedy 1989) which is a reasonable approximation over limited ranges of concentration and duration. TLVs do not explicitly consider sensitive subgroups; the TLV will be adjusted for continuous exposure and further decreased by a factor of 10, according to U.S. EPA procedure, to account for sensitive subgroups.

3.3.3. Acute RfD

Acute RfDs are conceptually similar to chronic RfDs. They are intended to represent levels over exposure that will not be associated with adverse effects in any member of the exposed population. As used in Forest Service risk assessments, acute RfD values are used to characterize risks associated with exposures lasting no longer than one day.

Acute RfD values for pesticides are relatively new. While the Office of Drinking Water has been deriving 1-day health advisories for many years (U.S. EPA/ODW 1990), the Office of Pesticides has only recently started using acute NOAEL values with recommended Margins of Exposure in the reregistration of pesticides and the development of pesticide tolerances under the Food Quality Protection Act (FQPA) (U.S. EPA/OPP 2005).

In Forest Service risk assessments, a surrogate acute RfD is typically derived by taking the NOAEL identified by the U.S. EPA/OPP and dividing the NOAEL by the recommended Margin of Exposure. Most often, the acute NOAEL is based on a multigeneration reproduction study. As noted in Section 3.1.9.2, these studies often involve exposures to two or three generations of rodents. This type of study is appropriate as the basis for an acute RfD under the assumption that any adverse reproductive effect could have occurred as the result of a brief period of time during the longer exposures involved in the multigeneration reproduction study. Less often, a NOAEL from a developmental (teratology) study may be used (Section 3.1.9.1.).

For some chemicals, the dose-duration relationship may be very weak; there may appear to be no substantial difference in the acute and longer-term or chronic toxicity of the chemical. As discussed in Section 3.1.3.3., this may occur for compounds that are rapidly eliminated and hence reach steady-state in a very brief period of time. For such compounds, no acute RfD is typically used and the consequences of acute exposures are assessed with the chronic RfD (Section 3.4).

3.3.4. Probit Analysis and Benchmark Doses

Many types of experiments used in both the human health and ecological risk assessments involve all-or-none responses such as mortality; the animal either lives or dies and there is no intermediate state. These types of responses are often termed *quantal* responses as opposed to *continuous* or *graded* responses such as changes in body weight. Continuous responses are often modeled in risk assessment using standard regression methods (Mendenhall and Scheaffer 1973). Quantal responses, however, require methods that are conceptually similar but methods that explicitly consider the variability and uncertainty in quantal responses that are associated with the binomial theorem (Finney 1971). While it is beyond the scope of this document to detail these methods, one particular method, probit analysis, is very important because it is often used in estimating LD₅₀ values (Section 3.1.4) and it forms the basis for benchmark doses, discussed below.

Probit analysis involves a general linear mode:

$$Pr = a + B d \quad (\text{Eq. 3-17})$$

where Pr is a transformation of the proportion responding (probits), d is the dose or a transformation of the dose such as \log_{10} dose, B is a slope or potency parameter (the relationship of dose to increasing response), and a is a measure of the background response (the response when dose is zero).

In the probit model, the underlying assumption is that the distribution of individual tolerances in a population is normally distributed with respect to dose or some transformation of dose. Thus, a probit is a normal equivalent deviate or essentially a standard deviation from a central response of 50% (Finney 1971). Occasionally, the logistic function may be used and this is based on the assumption that the proportion of responders, Pr , in the above equation is expressed as a logit, which is the logarithm of the ratio of the proportion of responders to the proportion of non-responders, often abbreviated as the $\log(p/q)$, where q is $1-p$.

Either the probit or logit models can be modified to consider additional explanatory variables (factors that influence the response), like duration of exposure expressed in some unit of time or a transformation of time (t):

$$Pr = a + B_1 d + B_2 t \quad (\text{Eq. 3-18})$$

The probit model, along with a number of other dose-response models, is being used with increasing frequency in benchmark dose calculations (U.S. EPA/ORD 2001). These calculations involve fitting the available dose-response data to an appropriate model, such as the probit model, and extrapolating the response down to some level, typically 5% or 10%, that is designated as the benchmark dose. The software provides an estimate of the lower confidence limit on the dose that is associated with this benchmark. The benchmark dose is then used rather than an experimental NOAEL to calculate the RfD. To date, this type of analysis has not been used in any completed Forest Service risk assessment, but the method is being applied by U.S. EPA in the development of newer RfDs (e.g., U.S. EPA 2004).

3.3.5. Dose-Response-Severity Relationships

For risk assessments in which the upper ranges of plausible exposures are below a level of concern (Section 3.4), only RfDs and comparable values are derived in the risk assessment. This approach is taken in order to make the risk assessments as simple as possible while maintaining an adequate expression of risk. If very conservative exposure assessments and very conservative dose-response assessments lead to no plausible basis for asserting that risks are likely, then no elaboration of the dose-response assessment is needed.

In some cases, however, some risks may be apparent and some attempt is made to further characterize the nature and severity of these risks based on the available dose-severity data. If data are sparse, this may involve little more than comparing the anticipated levels of exposure to LOAELs as well as to NOAELs. For pesticides with a richer data base, however, more elaborate dose-severity relationships may be constructed, either semi-quantitatively or quantitatively.

Semi-quantitative methods do not involve the use of an explicit dose-response model. In general, this will involve estimates of both animal doses and estimated human doses that might be associated with different types of adverse effects. An example of this type of analysis is presented in Table 3-6 for 2,4-D, a relatively well-studied compound. The animal doses are presented as ranges and the column labeled 'estimated human dose' is the animal dose divided by 10 (the uncertainty factor used for species to species extrapolation in deriving RfDs). The purpose of this is to provide dose estimates associated with effects of varying severity.

If adequate quantitative data are available, categorical regression may be used (Hertzberg and Miller 1985; Hertzberg 1989; McCullagh 1980). In addition to incorporating different endpoints and levels of severity, this method accommodates both quantal and continuous data. With categorical regression, it is also possible to incorporate additional explanatory (independent) variables, like exposure duration. Thus, this method can be used to estimate risk under various exposure scenarios.

Categorical regression assumes that each effect level can be associated with a distribution (e.g., normal or logistic) and that the shape of the distributions of the various severity levels are identical but shift to the right as severity and dose increase, as illustrated in Figure 3-3. Thus, at any given dose, the probability of observing an effect at a particular severity level can be estimated. Categorical regressions are conducted using a *link function*. That is, a logistic or probit distribution is used to describe quantitatively the variability of NOELs, NOAELs, AELs, and FELs (frank effect levels) illustrated in Figure 3-3.

An example of the application of categorical regression is given in Figure 3-4, again based on analysis of data on 2,4-D. At the RfD of 0.01 mg/kg/day, the probability of an adverse effect (AEL) is about 0.009 (9 in 1000). The probability of a frank effect being observed at the RfD would be about 0.00009 (9 in 100,000). While these estimates elaborate on the level of protection that may be afforded by the RfD, the method is most useful for expressing how risk might increase as the RfD is exceeded.

3.3.6. Carcinogenicity

Risk assessments conducted for the Forest Service do not typically include cancer risk assessments because the Forest Service does not use any pesticides that are classified as carcinogens. Nonetheless, some herbicides do contain potential carcinogens either as impurities in the technical grade active ingredient or as an impurity in an adjuvant. For example, as discussed in Section 3.1.15, hexachlorobenzene is a contaminant in two herbicides used by the Forest Service, clopyralid and picloram. In such a case, cancer potency factors derived by U.S.

EPA are used to estimate cancer risk. Details of the methods used are given by U.S. EPA (U.S. EPA/ORD 1996b, 2003). It should be noted that the U.S. EPA has not yet finalized these methods. Because the Forest Service defers to U.S. EPA in both the categorization of a compound as a carcinogen as well as the calculation of carcinogenic potency, the Forest Service risk assessments never directly derive cancer potency factors. These potency factors are often calculated based on the multistage model (Crump and Howe 1984), which is available in U.S. EPA's benchmark dose software (U.S. EPA/ORD 2001). If necessary, these methods could be employed in Forest Service risk assessments, but this situation has not arisen to date.

3.4. RISK CHARACTERIZATION

Risk characterization is the process of comparing the exposure assessment with the dose-response assessment to express the level of concern regarding a specific exposure scenario or set of scenarios (NRC 1983).

For systemic toxic effects, risk characterizations have been presented typically as either a Margin of Safety (MOS) or a Hazard Quotient (HQ). The Forest Service risk assessments generally use the HQ approach although the two methods are closely related.

A *margin of safety* is simply an experimental exposure level in animals, usually one that is not associated with adverse effects (i.e., *NOEL* or *NOAEL*), divided by an estimate of exposure (E_i):

$$\text{MOS} = \text{NOAEL} / E_i \quad (\text{Eq. 3-19})$$

Thus, as the exposure level decreases, the margin of safety increases.

A *hazard quotient* is the ratio of a projected level of exposure (E_i) divided by some index of an acceptable exposure or an exposure associated with a defined risk, such as an RfD. The RfD, in turn, is an experimental exposure level (i.e., *NOEL* or *NOAEL*) divided by an uncertainty factor (*UF*):

$$\text{HQ} = E_i / (\text{NOAEL}/\text{UF}) \quad (\text{Eq. 3-20})$$

Consequently, as the level of projected human exposure decreases, the hazard quotient decreases.

The obvious and trivial difference between these two methods is that they are inversely related to each other. The significant difference between the *margin of safety* and the *hazard quotient* approach, however, is that the *hazard quotient* method is based on an explicit uncertainty factor, dependent on the quality of the available data. The only time that these two methods will lead to differing interpretations of risk is when the acceptable margin of safety is set to a value other than the uncertainty factor used to derive the RfD, or when the assessments use different NOAELs. Otherwise, the two methods are equivalent.

RfDs are intended to be conservative estimates that incorporate a substantial margin between a dose that does not cause adverse effects and doses that cause adverse effects. This difference is referred to as a '*margin of protection*'. If the margin of protection is substantial, adverse effects may not be observed or even induced when the hazard index is greater than unity (i.e., exposure exceeds the *presumably safe* level). In order to assess the plausibility and nature of inducing or observing adverse effects, the relationship of exposed dose to the severity of effects is further considered, either qualitatively or quantitatively.

As with the dose-response assessments, the distinction between AELs and FELs is central to characterizing risk. When applied to risk characterizations, however, the distinction between

AELs and FELs may be subject to misinterpretation. Some, and perhaps most, of the exposure scenarios derived in a risk assessment may be associated with a low likelihood of an FEL based on the categorical regression analyses. In other words, no overt toxic effects are anticipated. This is not to be interpreted as suggesting that all of the exposure scenarios are acceptable or at least equally acceptable. Hazard indices may be exceeded by a substantial margin and may be in the region in which AELs are plausible. In such cases, humans subject to such exposures would probably be asymptomatic. Nonetheless, such individuals might experience subclinical changes that, if detected, would be regarded as justification for measures to reduce or eliminate the possibility of further exposure.

For carcinogenic effects, the risk associated with a given scenario and a single route of exposure (P_i) can be expressed simply as the potency factor (B) in units of (mg/kg/day⁻¹) multiplied by the exposure (E) in units of mg/kg/day:

$$P_i = B D \quad (\text{Eq. 3-21})$$

If more than one route of exposure is associated with a carcinogenic response or more than one source of exposure needed to be considered, the risks from all routes or sources can be added. A major source of uncertainty is introduced when the exposure duration for the scenario is substantially less than lifetime. This uncertainty cannot be quantified, but it is likely to result in underestimating risk if the compound affects early stages of the carcinogenic process.

In addition to these numerical expressions, the risk characterization section, more than any other part of the risk assessment, must explain the conclusions of the risk assessment in plain language. How this is done, specifically, depends largely on the nature of the perceived risk or the apparent lack of risk.

In some cases, a risk assessment may find no objective suggestion of an adverse effect based on the currently available data. In such cases, the risk characterization must clearly make the point that: ***Absolute safety cannot be proven and the absence of risk can never be demonstrated.*** No chemical is studied for all possible effects and the use of data from laboratory animals to estimate hazard or the lack of hazard to humans of other species is an uncertain process. Thus, prudence dictates that normal and reasonable care should be taken in the handling of any chemical. In other instances, risks may be apparent and this too must be clearly stated both quantitatively and qualitatively.

The risk characterization will also include a discussion of *biologically sensitive* subpopulations. Biological sensitivity here refers to a group of individuals or a subpopulation which for reasons of developmental stage or some other biological condition, are significantly more susceptible than the general population to a compound. Issues regarding individuals at greater risk because of increased exposure are addressed in the exposure assessment (Section 3.2).

Biologically sensitive subpopulations do not include individuals at the extreme lower end of a normal (uni-modal) distribution of tolerances. Conceptually, the welfare of those individuals should be incorporated into a uni-modal model in the dose-response assessment. Failure to differentiate among *biologically sensitive* subpopulations, sensitive individuals in a uni-modal population, and individuals at increased risk due to high levels of exposure may add a substantial amount of confusion to the risk assessment.

Some individuals may be atypically sensitive to chemical exposure because of their age (e.g., Calabrese 1986). Frequently, the very young or the very old are especially susceptible to the toxic effects of chemicals. Thus, everyone will belong to a high risk group at one or more times during their life span. Genetic factors, in contrast to developmental and aging factors, affect smaller subsections of the population. Some genetic conditions thought to predispose or enhance susceptibility to toxicants and the associated type of toxicant include cholinesterase variants (insecticides, cystic fibrosis), ozone and respiratory irritants, cystinosis and cystinuria (metals), glucose-6-phosphatase dehydrogenase deficiency (carbon monoxide), glutathione, glutathione peroxidase, and glutathione reductase deficiencies (lead and ozone), immunoglobulin A deficiency (respiratory irritants), immunological hypersensitivity (isocyanates), porphyrias (hexachlorobenzene, lead, barbiturates), sickle cell trait (aromatic amino and nitro compounds), and sickle cell anemia (carbon monoxide, cyanide). Furthermore, any genetic deficiency that results in altered xenobiotic metabolism may enhance susceptibility to chemicals.

Pre-existing disease states make individuals more susceptible to toxic chemicals. People with liver disease are less able than others to metabolize and detoxify foreign chemicals, and people with kidney disease are less able than others to excrete toxic chemicals. Carbon tetrachloride, PCBs, DDT, and other pesticides are among the chemicals that many people with liver disease may find more difficult to tolerate. People with kidney disease are especially sensitive to the effects of lead and other heavy metals. Asthma, chronic respiratory disease and heart disease predispose individuals to respiratory irritants, such as, nitrogen dioxide, ozone, sulfates, sulfur dioxide and carbon monoxide.

Behavioral and life style factors, such as smoking and alcohol and drug use not only increase an individual's exposure to toxic chemicals, but also increase the individual's susceptibility to other chemicals. Cigarette smoke itself contains a variety of carcinogens and other toxic chemicals. The chemicals in cigarette smoke may potentiate the toxicity of other pollutants. Alcohol and drug use enhance susceptibility to PCBs and pesticides by altering metabolizing enzyme systems in the liver. Dietary habits also may influence the toxicity of chemicals by producing changes in physiological and biochemical functions and nutritional status. Obese individuals may be more susceptible than others to toxic chemicals.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

This section of the risk assessment provides an overview of the available studies on the effects of the chemical to wildlife species. Depending on the nature of the available data, subsections for terrestrial species typically include mammals, birds, invertebrates, plants (macrophytes), and microorganisms. If justified by the amount and type of data, additional sub-groupings may be used. For aquatic species, subsections are typically provided on fish, amphibians, aquatic invertebrates, and aquatic plants (both macrophytes and algae). The hazard identification for wildlife mammals is usually based on the same information considered in the human health risk assessment, and this information is typically much more detailed than the information available on other groups because studies are often available on both lethal and sublethal effects. Data on the other groups is typically much less detailed. While information on sublethal effects are often available for some groups, much of the information consists of acute bioassays for lethality. This reflects a major conceptual difference between human health and ecological risk assessment. Human health risk assessment focuses on preventing the occurrence of any effect in any individual. Ecological risk assessment tends to focus on preventing adverse effects at the population level.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals – The hazard identification for terrestrial mammals is typically based on the discussion of the toxicity of the chemical to mammals that is given in the human health risk assessment (Section 3.1). There are two major additional considerations that may be discussed in this section: toxicity to canids (species related to dogs) and inferences on the toxicity of the chemical based on field studies.

Many of the pesticides used by the Forest Service, particularly the herbicides, are weak acids. Weak acids are often removed from the blood by the kidney, with eventual secretion in the urine. Part of this process involves active transport from the blood into kidney cells (e.g., Durkin et al. 2004). This active transport process in dogs is much less active than the active transport process in primates and other mammals (e.g., Timchalk and Nolan 1997). Consequently, dogs are less able to eliminate weak acids and may be substantially more sensitive to weak acids than other mammals. Thus, in risk assessments on weak acids, this section will emphasize any available information on the pharmacokinetics or toxicity of the compound in dogs relative to other mammalian species. If dogs appear to be more sensitive than other mammals, this may be considered further in the dose-response assessment (Section 4.3) and separate NOAEL or NOEC values may be derived for dogs. These values may then be used to characterize risks for other canid species that may be covered in the risk assessment – e.g., the consumption of a small mammal by a predator such as a coyote or wolf.

A consideration of field studies involving observations on terrestrial mammals may also be included in this section. While field studies involving mammals may be useful in the ecological

risk assessment, they are not typically considered or discussed in detail in the human health risk assessment. Field studies generally involve the application of a pesticide to a defined area and subsequent observations on wildlife populations. In this respect, field studies may be viewed as analogous to epidemiology studies that may be used in the human health risk assessment (Section 3.1.5.5). Like epidemiology studies, field studies may be difficult to interpret because the control site (an untreated area) may differ from the treated site. An additional complication with field studies is that some observed effects (e.g., changes in the composition of a population of mammals) may be due to secondary effects, such as changes in vegetation cover or changes in the availability of suitable prey species, rather than direct toxic effects. This is a common problem in the use of field studies for mammals as well as other groups, both terrestrial and aquatic, that are considered in the ecological risk assessment.

Other than considerations of canids and field studies, this section may emphasize some types of toxicity studies that are not used substantially in the human health risk assessment. The U.S. EPA/OPPTS (2005) does have a test guideline for acute dietary toxicity in wild mammals (OPPTS 850-2400). This type of bioassay is not typically required for herbicides but might be required for some other pesticides, particularly rodenticides. Unlike many of the other test guidelines given by EPA/OPPTS (2005), the OPPTS 850-2400 guideline does not recommend or require specific protocols or test species. A similar type of study is often available for rats and mice as part of standard subchronic or chronic toxicity studies (e.g., OPPTS 870.4100). In the design of these studies, shorter term dietary or drinking water range-finding studies, typically lasting 2 weeks, may be provided and may be used in assessing the consequences of acute dietary exposures.

As in the human health risk assessment, the results of various types of acute toxicity bioassays may be used to classify chemicals into various levels of toxicity – e.g., highly toxic to virtually nontoxic. The classification system currently used by U.S. EPA is summarized in Table 4-1. As with the corresponding classification scheme for human health effects (Table 3-2), Table 4-1 is only used in the hazard identification to categorize the pesticide and is not directly used in the risk characterization. As discussed in Section 1.2.1, all quantitative risk characterizations in Forest Service risk assessments are based on both a quantitative exposure assessment and quantitative dose-response assessment.

4.1.2.2. Birds – Information on the toxicity of pesticides to birds is typically much more limited than that for experimental mammals. While some toxicity studies on birds may be available in the open literature, most of the information is usually from studies required specifically by the U.S. EPA/OPPTS (2005) for the registration of pesticides: OPPTS 850-2100, avian acute oral toxicity; OPPTS 850-2200, avian acute dietary toxicity; and OPPTS 850-2300, avian reproductive toxicity.

The acute studies, both oral and dietary, most commonly involve tests on mallard ducks (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*). The acute oral study involves administration of the chemical either by gavage or capsule. Full studies will use at least five dose

levels, and the specific doses are selected based on the results of range-finding studies which typically use doses of 2, 20, 200, and 2,000 mg/kg bw. The birds receive a single dose and are observed for 14 days, although this period can be extended to 21-days if mortality is seen in the last 3 days of the normal 14-day observation period. Observations include mortality, food consumption, changes in body weight, signs of toxicity and gross examination of tissues after the animals are sacrificed. Histological examinations of tissue are usually not conducted. As with the mammalian oral study (Section 3.1.4), a limit test may be conducted at a single dose of 2,000 mg/kg. If no mortality occurs, the LD₅₀ value may be expressed as >2,000 mg/kg and no additional testing is required. As with the mammalian studies, the risk assessment will distinguish this type of information from studies in which some but less than 50% mortality occurred at the maximum dose.

The avian acute dietary toxicity study, OPPTS 850-2200, is similar to the acute oral study in general design and test species. Occasionally, however, other species may be used such as pigeon (*Columba livia*), Japanese quail (*Coturnix coturnix japonica*), ring-necked pheasant (*Phasianus colchicus*), and red-legged partridge (*Alectoris rufa*). The chemical is administered in the standard diet (laboratory chow) for a period of 5-days, and the birds are observed for an additional 3-days. This test is sometimes referred to as a 5-day dietary or 8-day dietary study, which can lead to some confusion if the duration of exposure is not clearly distinguished from the duration of observation. As with the acute oral study, the duration of observation can be increased up to 21 days if signs of toxicity are noted during the standard 3-day post-exposure observation period. As discussed further in Section 4.3, either the acute oral study or acute dietary study will often serve as the basis for an acute NOAEL or NOEC that is used in the dose-response assessment for birds.

Chronic studies in birds analogous to those conducted in mammals – i.e., studies that span a full or significant fraction of the animals life span – are almost never available. Typically, the consequences of longer-term exposure scenarios for birds are evaluated using the avian reproductive toxicity study, OPPTS 850-2300. These studies are generally conducted on mallard ducks or bobwhite quail. The compound is administered in the diet, and usually 3 concentrations plus a control group are used. The initial period of exposure is typically 6 to 8 weeks (42 to 56 days). After this period, egg laying is induced by manipulating the photoperiod over a 2 to 3 week period. After egg laying begins, the study is continued for an additional 8 to 10 weeks. During all three periods, dietary exposure is maintained and thus the total period of exposure is 16 weeks (112 days) to 21 weeks (147 days). If adverse effects are noted, a withdrawal period of up to 3 weeks may be incorporated into the study. Observations on adult birds include those normally done in the acute studies – food consumption, body weight changes, and signs of toxicity. Egg production, egg hatching, and the viability of offspring are also assayed. As in the acute studies, gross pathological examinations are conducted on all birds but histopathology (microscopic examination of tissue) is not typically done.

Other data on birds may include field studies. If such data are available and are relevant to the hazard identification, the data are detailed in this section. The limitations on the use of field data

are generally similar to those encountered in the use of field data on experimental mammals (Section 4.1.2.3).

4.1.2.3. Terrestrial Invertebrates – There is substantial variability in the types of information that are available on terrestrial invertebrates. For herbicides, the type of pesticide on which most Forest Service risk assessments have been conducted, only relatively simple and standard bioassays may be available: the honey bee acute contact toxicity (OPPTS 850.3020), the honey bee toxicity of residues on foliage (OPPTS 850.3030), and the earthworm subchronic toxicity test (OPPTS 850.6200).

The acute contact toxicity study in honey bees is often the only kind of invertebrate toxicity study available on herbicides. This acute study is similar in design to acute toxicity studies conducted on mammals and birds but involves direct application, either by micro-applicator (topical drop) or whole body exposure. For herbicides and other compounds that are generally thought to be nontoxic to invertebrates, only a limit test may be conducted and this is typically done at a dose of 25 micrograms per bee. In many herbicide risk assessments, a standard body weight of 0.093 g for the honey bee (USDA/APHIS. 1993) is used to convert the dose per bee to a mg/kg bw dose – i.e., 0.025 mg/0.000093 kg or about 270 mg/kg bw. If mortality is observed in the limit test, then standard range-finding and full studies may be conducted.

The earthworm toxicity test (OPPTS 850.6200) involves exposing a species of earthworm, typically *Eisenia fetida*, to various concentrations of the test compound in soil for a period of 28-days. The use of limit tests are not discussed in the OPPTS protocol. Range-finding studies are conducted as 0.1, 1.0, 10, 100, 1,000 mg/kg dry weight artificial soil. This type of test is not consistently required for the registration of pesticides, particularly herbicides, and is typically a greater concern in the potential effects of industrial chemicals that may be disposed of in landfills.

Earthworms and honeybees comprise only a very small fraction of the terrestrial invertebrates that may be found in any habitat. For insecticides, there are typically a very large number of additional laboratory toxicity studies as well as field studies in both target and nontarget invertebrate species, and the summary of effects and the hazard identification for terrestrial invertebrates will be much more elaborate.

4.1.2.4. Terrestrial Plants (Macrophytes) – The available data on terrestrial plants is virtually the mirror image of the available data on terrestrial invertebrates in terms of the relative amount and complexity of the information for herbicides and insecticides (as well as other biocides such as rodenticides). For insecticides and other biocides, virtually no information may be available and there may be no plausible basis for anticipating any adverse effects in plants. For some non-herbicidal pesticides that are applied directly to plants, Tier I studies may be required by the U.S. EPA for both seedling emergence (OPPTS 850.4100) and vegetative vigor (OPPTS 850.4150).

Studies on seedling emergence and vegetative vigor are the two basic types of bioassays that are covered and used in Forest Service risk assessments. Seedling emergence studies typically involve soil exposure and vegetative vigor studies typically involve direct spray. The former are used to characterize risk associated with soil contamination by runoff, and the latter are used to characterize risks associated with direct spray or spray drift.

Seedling emergence studies, as the name implies, involves the treatment of seeds with the chemical. The test is conducted by applying the chemical directly to the soil or directly to seeds and then observing whether or not germination occurs. In the Tier 1 study, three required species are corn, soybeans, and a root crop as well as seven other species, usually tomato, cucumber, lettuce, cabbage, oat, ryegrass, and onion. The test requires six species of at least four families of dicots – e.g., herbaceous plants – and four species of at least two families of monocots – e.g., grasses. The Tier II assay (OPPTS 850.4225) requires at least 10 species of plants. A required dicot species is soybean (*Glycine max*) and a required monocot is corn (*Zea mays*). At least one other dicot must be a root crop such as carrot (*Ducus carrota*), onion (*Allium cepa*), beet (*Beta vulgaris*), or sugarbeet (*Beta vulgaris*). Other species which are commonly included in the test include tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativa*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), oat (*Avena sativa*), and perennial ryegrass (*Lolium perenne*). The duration of the bioassay is typically about 14 days during which time the number of emerged seedlings, shoot heights, and signs of visual phytotoxicity are recorded as well as other endpoints such as root dry weight. Results are typically reported as NOEC (or EC₅) and LOEC values as well as estimates of the EC₂₅ and EC₅₀.

Vegetative vigor studies, either Tier 1 (OPPTS 850.4150) or Tier II (OPPTS 850.4250), involve direct foliar application 2 to 4 weeks after the plants have emerged. The species tested are similar to those used in seedling emergence studies. Also as with the seedling emergence studies, the Tier I vegetative vigor study is analogous to a limit test in which the limit is defined as 3 times the maximum labeled application rate. In the Tier II test, at least 5 application rates are tested and the endpoints reported include measures of growth such as dry shoot weight, dry root weight, shoot height, and visual signs of phytotoxicity, with an observation period of at least 14-days. In the Tier II bioassay, the results are typically reported as in the Tier II seedling emergence study – i.e., the NOEC (or EC₅) and LOEC values as well as estimates of the EC₂₅ and EC₅₀.

For herbicides, a large number of field studies as well as other more specialized laboratory bioassays may be available. The field studies have the same general limitations as those discussed in Section 4.1.2.1. Most importantly, any effective herbicide will kill at least some species of plants and this will alter both the sunlight and nutrients available to other competing plants. An attempt is made in the discussion of the risk assessment to distinguish these types of secondary effects from direct toxic effects. In addition, field studies often include studies on the effects of the herbicide on various target species. These types of studies are referred to as efficacy studies and are generally not covered in detail in the risk assessment because the focus of this section of the risk assessment is on nontarget plants.

4.1.2.5. Terrestrial Microorganisms – Studies on the toxicity of pesticides, particularly the herbicides, are commonly available in both the published literature as well as the standard studies that may be submitted to U.S. EPA in support of pesticide registration. The latter group of studies include rhizobium-legume toxicity (OPPTS 850.4600) which focus on soil microorganisms that are associated with roots of plants (i.e., legumes), more general assays of soil microbial communities (OPPTS 850.5100), and soil-core sample microcosm assays (OPPTS 850.2450). The observations made in these microbial assays typically include soil microbial populations (which may or may not be broken down by type of microorganism) as well as various assays of microbial activity such as CO₂ production and NO₃ and NH₃ concentrations. A variety of other endpoints (e.g., rates of formation or decomposition of various compounds such as cellulose, protein or starch) may be reported in the open literature. The soil microcosm assays (OPPTS 850.2450) and similar studies reported in the open literature may also include information on effects on soil invertebrates. If so, these studies are also discussed in Section 4.1.2.3.

The assays on microbial toxicity submitted directly to U.S. EPA for registration involve soil exposures as these are directly relevant to the risk assessment. Many microbial toxicity studies in the open literature involve pure cultures of microorganisms in artificial media such as agar or liquid culture. These types of assays are less directly relevant and are clearly distinguished from soil assays in the risk assessment.

4.1.3. Aquatic Organisms

4.1.3.1. Fish – Three general types of relatively standardized studies may be available on fish: acute toxicity studies (e.g., OPPTS 850-1075); egg-and-fry studies, also referred to as early life-stage studies (e.g., OPPTS 850-1400); and full life cycle studies (e.g., OPPTS 850-1500).

Freshwater species that are commonly used in acute assays submitted to the U.S. EPA and preferred by the U.S. EPA include rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*). A large number of other species may be used, including Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), coho salmon (*Oncorhynchus kisutch*), carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), guppy (*Poecilia reticulata*), red killifish (*Oryzias latipes*), threespine stickleback (*Gasterosteus aculeatus*), and zebrafish (*Brachydanio rerio*). A number of saltwater species may also be tested, including Atlantic silverside (*Menidia menidia*), tidewater silverside (*Menidia peninsulae*), and sheepshead minnow (*Cyprinodon variegatus*). Typically, Forest Service applications will not involve applications near salt water and exposure assessments for saltwater species are not generally conducted. Information on saltwater species, however, is included and these species may be used to identify the most sensitive species for the dose-response assessment (Section 4.3).

The design of the acute toxicity bioassays is similar to the design of other acute toxicity bioassays. Range-finding studies as well as limit assays may be used. The common limit concentration is 1000 mg/L – if less than half of the fish die at a concentration of 1000 mg/L,

further testing may not be required and the LC₅₀ value may be reported as >1000 mg/L. Like all similar values, the risk assessment will distinguish such values in terms of the percent mortality observed. A full study generally involves at least five concentrations with 14 to 20 fish per concentration. As with other acute studies, partial mortality is needed in at least two concentrations in order to perform the probit analysis. LC₅₀ (and sometimes other response rates) values are typically reported for 24, 48, and 96 hours, and differences between these values – progressively lower LC₅₀ values as time increases – may indicate either accumulation or cumulative damage (Section 3.1.3). In Forest Service risk assessments, NOEC and LOEC values are reported if available. As discussed further in Section 4.3 and Section 4.4, the U.S. EPA will typically use an LC₅₀ value for risk characterization while the Forest Service prefers to use an NOEC for sublethal effects.

Early life-stage studies in fish (OPPTS 850-1400) are analogous to mammalian teratology studies (Section 3.1.9.1.). The test involves exposing fertilized eggs to various concentrations of the chemical and maintaining the exposure until the fish are free-feeding. Freshwater species commonly used in this assay include rainbow trout, fathead minnow, zebra fish, and ricefish (*Oryzias latipes*). The sheepshead minnow is the only saltwater species that is typically used. Typically, five concentrations are tested along with a control group. If a vehicle is used, a vehicle control is also included. The primary observations are of hatching of the eggs and survival of the fry, gross abnormalities (terata), behavior, length and weight. Results are typically reported as NOEC and LOEC values using a *p*-value of 0.05 to define the NOEC. This type of bioassay is elaborate and expensive. While these types of studies are funded by registrants as part of the registration process and while these studies may occasionally be published in the open literature, egg-and-fry studies are not typically conducted by other researchers and are not typically published. While these studies are not true chronic studies, they are often the only longer-term study available on a presumably sensitive life-stage, and these studies often serve as the basis for the longer-term dose-response assessment in fish.

Fish life cycle toxicity studies (OPPTS 850.1500) involve essentially egg-to-egg exposures. As with the egg-and-fry study, the life cycle study starts with fertilized eggs. The study, however, continues throughout the life of the initial generation, analogous to the P (parental) generation in a multigeneration reproduction study in mammals, and continues until the P generation produces eggs. This type of test is almost always conducted on either the fathead minnow (freshwater) or the sheepshead minnow (estuarine). This type of test is very expensive and is required only when the chemical is intended to be applied directly to water or when the ambient concentrations of the chemical are expected to be equal to or greater than one-tenth of the no-effect level in the fish early life-stage or invertebrate life-cycle test. When available, these tests are used for assessing the consequences of longer-term exposures unless egg-and-fry studies on other species appear to be more sensitive indicators of risk – i.e., have lower NOEC values.

The above types of tests are the studies most commonly used in Forest Service risk assessments. Fish, however, are very common test species and a large number of different types of species and types of assays may be available in the open literature. These studies can be highly variable in

the species tested, protocols used, and endpoints examined. Studies from the open literature reporting adverse effects in fish are always included in the risk assessment, at least in the appendices. The extent to which non-standard studies may be used to quantitatively modify the dose-response assessment or risk characterization are addressed on a case-by-case basis in each risk assessment. In general, Forest Service risk assessments will use non-standard studies if they suggest a greater risk than standard studies and if the reported endpoints can be related plausibly to adverse effects that might be seen in populations of fish in the environment.

Lastly, field studies that include observations on fish are occasionally available as well as mesocosm (e.g., littoral enclosure) studies. These studies are used to the extent possible as a check on the available laboratory toxicity studies. The general limitations on field studies (Section 4.1.2.1) apply to observations from field studies that involve fish. Better controlled mesocosm studies are generally more useful in assessing the relevance of standard laboratory studies to potential hazards in the field.

4.1.3.2. Amphibians – Amphibians (e.g., frogs, salamanders, and toads) are cold-blooded animals that spend time both on land and in water but breeding and development typically occur in water. Thus, most Forest Service risk assessments contain only a single section on amphibian that is included in the major sections on aquatic organisms. Occasionally, if data are available on both terrestrial exposures and effects on amphibians from terrestrial exposures, a subsection on amphibians may be included as part of Section 4.1.2 (Terrestrial Organisms).

Although the amount of information on the toxicity of pesticides to amphibians is increasing (e.g., Sparling et al. 2000), very little toxicity data are generally available on amphibians compared to other aquatic species. The most commonly available study is the Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX) bioassay (e.g., Fort et al. 2004). This study typically involves exposing frog embryos to the test chemical for a 96 hour period. The study is similar in design to acute toxicity study in fish in terms of the number of concentrations and reporting of results. The endpoints include observations of mortality as well as malformations (NTP 2000).

The U.S. EPA (2005) has a general protocol (OPPTS 850.1800) for a 30-day subchronic sediment toxicity study using bullfrog tadpoles (*Rana catesbeiana*). This type of study is similar in design to other aquatic bioassays.

Because of the relative sparsity of data available on toxic effects to amphibians and the high level of concern with effects on amphibians because they may be good indicator species, any available information on effects to amphibians are typically reviewed in some detail. If the data are sufficient, these data are used in the dose-response assessment (Section 4.3).

4.1.3.3. Aquatic Invertebrates – Many aquatic invertebrates are relatively simple organisms to culture and test in aquatic toxicity studies, and standard acute toxicity protocols from U.S. EPA/OPPTS (2005) are available on a number of invertebrate species: daphnids (OPTTS 850.1010), gammarids (OPTTS 850.1020), oysters (OPTTS 850.1025), mysid shrimp (OPTTS

850.1035), penaeid shrimp (OPTTS 850.1045), and several species of bivalves (OPTTS 850.1055). These tests are similar in design to acute toxicity studies in fish (Section 4.1.3.1), although some may involve somewhat shorter periods of exposure – e.g., the daphnid study typically only lasts for 48 hours. Acute toxicity studies will often be available in the open literature as well and may be conducted on a large number of different species, although the overall designs of most studies are similar to those (and often follow) standard protocols from either the U.S. EPA or the European Organization for Economic Co-operation and Development (OECD).

Chronic studies on invertebrates are generally limited to daphnids (OPTTS 850.1300 for a freshwater species) or mysid shrimp (OPTTS 850.1350 for a saltwater species). These are true chronic studies. Although the daphnid study is typically conducted for only 21-days, the short life span and rapid reproductive capacity of daphnids may result in exposures to several generations of young. The most common test species is *Daphnia magna* although *D. pulex* are sometimes used. The chronic daphnid study is typically the only study available on the chronic toxicity of a pesticide to freshwater invertebrates.

As with fish, field studies or mesocosm studies on herbicides that include observations on invertebrates are occasionally available and these studies may be used to supplement the dose-response assessment. For insecticides, field studies may be abundant and of sufficiently high quality to justify using these studies rather than laboratory studies as the basis for the dose-response assessment. This is considered and discussed on a case-by-case basis in each risk assessment.

4.1.3.4. Aquatic Plants – Aquatic plants comprise both macrophytes (large multicellular plants) and algae (small microscopic plants). Bioassays in aquatic algae typically involve freshwater green alga (*Selenastrum capricornutum* or *Raphidocelis subcapitata*), a freshwater diatom (*Navicula pelliculosa*), a marine diatom (*Skeletonema costatum*), and a blue-green alga or cyanobacterium (*Anabaena flos-aquae*). Bioassays on macrophytes typically use a species of duck weed (e.g., *Lemna gibba*). The duration of exposure for algae is typically 48-hours and the duration for duckweed is typically about 7-days. Both types of studies measure growth (either as cell count or gross weight) and express results as effective concentrations (e.g., EC₅₀) rather than lethal concentrations (e.g., LC₅₀). As with most other types of bioassays, the studies often report NOEC and LOEC values, and NOEC values are typically used in the dose-response assessment.

Field studies may be relatively abundant for some herbicides, particularly for those that are intended for aquatic weed control. These studies may be directly useful in the dose-response assessment as long as concentrations in water are reported.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment, the specific exposure scenarios that are considered in this section are determined by the application method and the chemical and toxicological properties of the compound. Generally, this section will consider acute and longer-term durations of oral exposure (food or drinking water) as well as soil contamination, a direct spray, and drift. The exposure assessment for aquatic species typically relies on the estimated peak and longer-term concentrations in water that are used in the human health risk assessment as well as the exposure assessments for terrestrial wildlife from the consumption of contaminated water. Similarly, exposures of soil organisms to a pesticide are typically based on the GLEAMS modeling that is used to estimate concentrations in water and/or available monitoring data. Exposures to terrestrial plants are estimated both as concentrations in soil and direct foliar contamination either from direct spray or drift. For some species of terrestrial animals (typically insects), standard toxicity studies may report units that are not readily converted to mg agent/kg body weight. For example, some contact toxicity studies express exposure only in mass of agent per unit surface area – e.g., lb/acre or mg/m². In such a case, some dose-response assessments may be based on units of mass of agent per unit surface area and the exposure assessment is simply expressed as the application rate, or some fraction of the application rate to account for drift.

The exposure scenarios for terrestrial animals are similar in many respects to the exposure scenarios used in the human health risk assessment. Terrestrial animals might be exposed to any applied insecticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. Estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. For dermal exposures to terrestrial animals, the units of measure usually are expressed in mg of agent per cm² of surface area of the organism and abbreviated as mg/cm². In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm² and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

As with the human health exposure assessment, the computational details for each exposure assessment are presented in worksheets. Given the large number of species that could be exposed to insecticides and the varied diets in each of these species, a very large number of different exposure scenarios could be generated. For the generic risk assessments, an attempt is made to limit the number of exposure scenarios. The specific exposure scenarios presented in the general risk assessments are designed as conservative screening scenarios that may serve as guides for more detailed site-specific assessments by identifying the groups of organisms and routes of exposure that are of greatest concern.

4.2.2. Terrestrial Animals

Because of the relationship of body weight to surface area and to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, than large animals will receive for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or bird. For mammals, the body weight is taken as 20 grams (typical of mice), and exposure assessments are conducted for direct spray, and consumption of contaminated fruit and contaminated water. Grasses will generally have higher concentrations of insecticides than fruits and other types of vegetation (Fletcher et al. 1994; Hoerger and Kenaga 1972). Although many small mammals do not generally consume large amounts of grass, many have a diet of mixed grasses and other herbaceous plants. In order to consider the potential consequences of the higher residues in grass, scenarios for the assessment of contaminated grass are based on both a small and a large mammal.

Other exposure scenarios for mammals involve the consumption of contaminated insects by a small mammal and the consumption by a large mammalian carnivore of small mammals contaminated via direct spray. Exposure scenarios for birds involve the consumption of contaminated insects by a small bird, the consumption of contaminated grasses by a large bird, the consumption of contaminated fish by a predatory bird, the consumption by a predatory bird of small mammals contaminated via direct spray. Additional exposure scenarios may be elaborated depending on how the pesticide is used and applied.

4.2.2.1. Direct Spray – In the broadcast application of any insecticide, wildlife species may be sprayed directly. This scenario is similar to the accidental exposure scenarios for the general public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, three groups of direct spray exposure assessments are conducted. The first scenario involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. The range of application rates as well as the typical application rate is used to define the amount deposited on the organism. Typically, the absorbed dose over the first day (i.e., a 24-hour period) is estimated using the assumption of first-order dermal absorption rate that is derived in the human health risk assessment (Section 3.1.3.2). Occasionally, first-order dermal absorption rates will be available for rodents or other mammalian species. If so, these values are used rather than the estimated first-order dermal absorption rate given in the human health risk assessment. An empirical relationship between body weight and surface area (Boxenbaum and D'Souza 1990) is used to estimate the surface area of the animal. The estimates of absorbed doses in this scenario may bracket plausible levels of exposure for small mammals based on uncertainties in the dermal absorption rate.

Other, perhaps more substantial, uncertainties affect the estimates for absorbed dose. For example, the estimate based on first-order dermal absorption does not consider fugitive losses from the surface of the animal and may overestimate the absorbed dose. Conversely, birds,

mammals, and other animals may groom frequently and grooming may contribute to the total absorbed dose by direct ingestion of the compound residing on fur or feathers. Other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals. Quantitative methods for considering the effects of grooming or increased dermal permeability are not available. As a conservative upper limit, a second exposure scenario is typically given in which complete absorption of the deposited dose over day 1 of exposure is assumed.

Because of the relationship of body size to surface area, very small organisms, like bees and other terrestrial invertebrates, might be exposed to much greater amounts of a pesticide per unit body weight compared with small mammals. Consequently, a third exposure assessment is typically developed using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993) and the equation above for body surface area proposed by Boxenbaum and D'Souza (1990). For most compounds, no information will be available regarding the dermal absorption rate in bees or other invertebrates. Thus, this exposure scenario will generally assume complete absorption over the first day of exposure. As noted above, exposures for some terrestrial invertebrates are based on toxicity studies or field studies in which application rate or some fraction of the application rate is the most relevant expression of exposure. These instances are detailed and discussed in this section.

Direct spray scenarios are not generally given for large mammals. As noted above, allometric relationships dictate that large mammals will be exposed to lesser amounts of a compound in any direct spray scenario than smaller mammals. However, in cases where the toxicity data indicate that large mammals are more sensitive than small mammals (see Section 4.3), direct spray scenarios for larger mammals may be given.

4.2.2.2. Indirect Contact – As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment in which transfer rates for humans are available, there are generally no transfer rates available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5 to 1-hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Compared to humans, wildlife are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation, although there are no data regarding the kinetics of such a process.

For highly lipophilic compounds – i.e., compounds with a low water solubility and a high $k_{o/w}$ – it is plausible that the absorbed dose resulting from contact with contaminated vegetation will be as great as those associated with comparable direct spray scenarios, and possibly larger than those associated with the consumption of contaminated vegetation. For hydrophilic compounds – i.e., compounds with a high water solubility and a low $k_{o/w}$ – the compound is not likely to

partition from the surface of contaminated vegetation to the surface of skin, feathers, or fur. Thus, a plausible partition coefficient is unity (i.e., the concentration of the chemical on the surface of the animal will be equal to the dislodgeable residue on the vegetation). Under these assumptions, the absorbed dose resulting from contact with contaminated vegetation might be on the order of one-tenth that associated with comparable direct spray scenarios. All of these assumptions, however, are speculative and are not generally used to quantify exposures in the risk assessments. Thus, the potential for effects from contact with contaminated vegetation is only qualitatively addressed.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – Many pesticides used by the Forest Service are applied directly to vegetation. Consequently, the consumption of contaminated vegetation is an obvious concern and separate exposure scenarios are developed for acute and chronic exposure scenarios for a small and a large mammal as well as large birds. If a compound may be applied more than once per season, the impact of multiple applications are considered using the same methods as in the human health risk assessment (Section 3.2.3.6).

For the consumption of contaminated vegetation, a small mammal is used because allometric relationships indicate that small mammals will ingest greater amounts of food per unit body weight, compared with large mammals. The amount of food consumed per day by a small mammal (an animal weighing approximately 20 g) is equal to about 15% of the mammal's total body weight (U.S. EPA/ORD 1989). When applied generally, this value may overestimate or underestimate exposure in some circumstances. For example, a 20 g herbivore has a caloric requirement of about 13.5 kcal/day. If the diet of the herbivore consists largely of seeds (4.92 kcal/g), the animal would have to consume a daily amount of food equivalent to approximately 14% of its body weight $[(13.5 \text{ kcal/day} \div 4.92 \text{ kcal/g}) \div 20\text{g} = 0.137]$. Conversely, if the diet of the herbivore consists largely of vegetation (2.46 kcal/g), the animal would have to consume a daily amount of food equivalent to approximately 27% of its body weight $[(13.5 \text{ kcal/day} \div 2.46 \text{ kcal/g}) \div 20\text{g} = 0.274]$ (U.S. EPA/ORD 1993, pp.3-5 to 3-6). For this exposure assessment (Worksheet F03), the amount of food consumed per day by a small mammal weighing 20 g is estimated at about 3.6 g/day or about 18% of body weight per day from the general allometric relationship for food consumption in rodents (U.S. EPA/ORD 1993, p. 3-6).

A large herbivorous mammal is included because empirical relationships of concentrations of pesticides in vegetation, discussed below, indicate that grasses may have substantially higher pesticide residues than other types of vegetation such as forage crops or fruits. Grasses are an important part of the diet for some large herbivores but most small mammals do not consume grasses as a substantial proportion of their diet. Thus, even though using residues from grass to model exposure for a small mammal is the most conservative approach, it is not generally applicable to the assessment of potential adverse effects. Hence, in the exposure scenarios for large mammals, the consumption of contaminated range grass is modeled for a 70 kg herbivore. Caloric requirements for herbivores and the caloric content of vegetation are used to estimate food consumption based on data from U.S. EPA/ORD (1993). As with all of the other exposure scenarios, details of these exposures are given in detailed worksheets.

For the acute exposures, the assumption is made that the vegetation is sprayed directly and that 100% of the animal's diet is contaminated – i.e., the animal grazes on site. While appropriately conservative for acute exposures, neither of these assumptions are plausible for longer-term exposures. Thus, for the longer-term exposure scenarios for the large mammal, two sub-scenarios are given. The first is an on-site scenario that assumes that a 70 kg herbivore consumes short grass for a 90 day period after application of the chemical. In the worksheets, the contaminated vegetation is assumed to account for 30% of the diet with a range of 10% to 100% of the diet. These are essentially arbitrary assumptions reflecting grazing time at the application site by the animal. Because the animal is assumed to be feeding at the application site, drift is set to unity – i.e., direct spray. The second sub-scenario is similar except the assumption is made that the animal is grazing at distances of 25 to 100 feet from the application site (decreasing risk), but that the animal consumes 100% of the diet from the contaminated area (increasing risk). For this scenario, AgDRIFT (Teske et al. 2001) is used to estimate deposition on the off-site vegetation. This model is discussed further below in Section 4.2.3.2.

The consumption of contaminated vegetation is also modeled for a large bird. For these exposure scenarios, the consumption of range grass by a 4 kg herbivorous bird, such as a Canada Goose, is modeled for both acute and chronic exposures. As with the large mammal, the two chronic exposure scenarios involve sub-scenarios for on-site as well as off-site exposure.

For this component of the exposure assessment, the estimated amounts of pesticide residue in vegetation are based on the relationship between application rate and residue rates on different types of vegetation. These residue rates are typically based on estimated residue rates from Fletcher et al. (1994). If chemical-specific residue rates are available and are substantially different from those given by Fletcher et al. (1994), the chemical-specific residue rates may be used. This will typically be done if the chemical-specific residue rates are higher than those given by Fletcher et al. (1994).

Similarly, the consumption of contaminated insects is modeled for a small (10g) bird and a small (20g) mammal. Most often, no monitoring data will be available on the concentrations of the compound in insects after defined applications. In these cases, the empirical relationships recommended by Fletcher et al. (1994) are used as surrogates. In most instances, the residue rates from small insects are used – 45 to 135 ppm per lb/ac – rather than the residue rates from large insects – 7 to 15 ppm per lb/ac.

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal or a predatory bird. Each of these scenarios assumes that the small mammal is directly sprayed at the specified application and the concentration of the compound in the small mammal is taken from the scenario for the direct spray of a small mammal under the assumption of 100% absorption.

In addition to the consumption of contaminated vegetation and insects, pesticides may contaminate ambient water and fish. Thus, a separate exposure scenario is developed for the

consumption of contaminated fish by a predatory bird in both acute and chronic exposures. Because predatory birds usually consume more food per unit body weight than do predatory mammals (U.S. EPA 1993, pp. 3-4 to 3-6), separate exposure scenarios for the consumption of contaminated fish by predatory mammals are not typically developed.

4.2.2.4. Ingestion of Contaminated Water – Estimated concentrations of pesticide in water are identical to those used in the human health risk assessment. The only major differences involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA 1989). Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (0.25 L/kg body weight/day). These values are used in the exposure assessment for the small (20 g) mammal. Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the estimate of the ingested dose include the field dilution rates (the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons. In the exposure scenario involving contaminated ponds or streams due to contamination by runoff or percolation, the factors that affect the variability are the water contamination rate (see Section 3.2.3.4.2) and the application rate.

4.2.3. Terrestrial Plants

As noted above, most pesticides used by the Forest Service are applied directly to vegetation. Thus, terrestrial plants will certainly be exposed to these pesticides. A large number of different exposure assessments could be made for terrestrial plants – i.e., direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Such exposure assessments are typically conducted for herbicides. For other pesticides, however, the development of such exposure assessments would serve no purpose because there is no basis for asserting that adverse effects in terrestrial plants are plausible. Thus, no formal exposure assessments for non-herbicidal pesticides are typically conducted for terrestrial plants, and the following discussion of specific exposure scenarios are typically conducted only for herbicides.

4.2.3.1. Direct Spray – Unintended direct spray will result in an exposure level equivalent to the application rate. For many types of herbicide applications – e.g., rights-of-way management – it is plausible that some non-target plants immediately adjacent to the application site could be sprayed directly. This type of scenario is modeled in the human health risk assessment for the consumption of contaminated vegetation and is typically considered for terrestrial plants.

4.2.3.2. Drift – Because off-site drift is more or less a physical process that depends on droplet size and meteorological conditions rather than the specific properties of the herbicide, estimates of off-site drift can be modeled using AgDRIFT (Teske et al. 2001). AgDRIFT is a model developed as a joint effort by the EPA Office of Research and Development and the Spray Drift Task Force, a coalition of pesticide registrants. AgDRIFT is based on the algorithms in FSCBG (Teske and Curbishley, 1990), a drift model previously used by USDA.

For aerial applications, AgDRIFT permits very detailed modeling of drift based on the chemical and physical properties of the applied product, the configuration of the aircraft, wind speed, and temperature. For ground applications, AgDRIFT provides estimates of drift based solely on distance downwind as well as the types of ground application: low boom spray, high boom spray, and orchard airblast. AgDRIFT gives a detailed evaluation of a very large number of field studies and is likely to provide more reliable estimates of drift. For ground broadcast applications, applications will typically involve low boom ground spray and these estimates from AgDRIFT are used. Representative estimates based on AgDRIFT (Version 1.16) are given in the worksheets. The AgDRIFT estimates are used for consistency with comparable exposure assessments conducted by the U.S. EPA. Further details of AgDRIFT are available at <http://www.AgDRIFT.com/>.

Drift associated with backpack applications (directed foliar applications) are likely to be much less. Few studies are available for quantitatively assessing drift after backpack applications, and drift from backpack applications are not currently made in the Forest Service risk assessments. Ando et al. (2003) have published some information that may be useful for quantitatively estimating drift from backpack applications. This study was funded under a grant from the Forest Service and additional data (not included in the publication) from this study has been provided by the Forest Service. These data along with any additional data that is encountered may be useful in quantifying backpack drift and these data may be incorporated into future revisions of the current document.

4.2.3.3. *Runoff* – Any pesticide may be transported to off-site soil by runoff or percolation. Both runoff and percolation are considered in estimating contamination of ambient water. Only runoff is considered in assessing off-site soil contamination. This approach is reasonable because off-site runoff will contaminate the off-site soil surface and could impact non-target plants. Percolation, on the other hand, represents the amount of the herbicide that is transported below the root zone and thus may impact water quality but should not affect off-site vegetation. The GLEAMS modeling used to estimate concentrations in water (Section 3.2.3.4.2) provides data on loss by runoff. These data are typically modeled for clay, loam, and sand at rainfall rates ranging from 5 inches to 250 inches per year. These data may be used in addition to any available monitoring studies that provide estimates of runoff after defined applications.

4.2.3.4. *Contaminated Irrigation Water* – Unintended direct exposures of nontarget plant species may occur through the use of contaminated ambient water for irrigation. The effects of exposure to contaminated irrigation water on nontarget vegetation have been observed for some herbicides (e.g., Bhandary et al. 1991).

The levels of exposure associated with this scenario will depend on the concentration of the pesticide in the ambient water used for irrigation and the amount of irrigation water that is applied. Concentrations in ambient water are generally based on the concentrations modeled in the human health risk assessment (Section 3.2.3.4). The amount of irrigation water that may be applied will be highly dependent on the climate, soil type, topography, and plant species under

cultivation. Thus, the selection of an irrigation rate is somewhat arbitrary. Typically, plants require 0.1 to 0.3 inch of water per day (Delaware Cooperative Extension Service 1999). In the absence of any general approach of determining and expressing the variability of irrigation rates, the application of one inch of irrigation water is used in the risk assessments. This is somewhat higher than the maximum daily irrigation rate for sandy soil (0.75 inches/day) and substantially higher than the maximum daily irrigation rate for clay (0.15 inches/day) (Delaware Cooperative Extension Service 1999). This variability may be addressed further in the risk characterization if risks are apparent (Section 4.4).

4.2.3.5. Wind Erosion – Wind erosion of soil is a major transport mechanism for soil (e.g., Winegardner 1996) and this mechanism has been associated with the environmental transport of herbicides (Buser 1990). Numerous models have been developed for wind erosion (e.g., Strek and Spaan 1997; Strek and Stein 1997) and the quantitative aspects of soil erosion by wind are extremely complex and site-specific. Field studies conducted on agricultural sites found that wind erosion may account for annual soil losses ranging from 2 to 6.5 metric tons/ha (Allen and Fryrear 1977). The upper range reported by Allen and Fryrear (1977) is nearly the same as the rate of 2.2 tons/acre (5.4 tons/ha) recently reported by the USDA (1998). The temporal sequence of soil loss (i.e., the amount lost after a specific storm event involving high winds) depends heavily on soil characteristics, and meteorological and topographical conditions.

To estimate the potential transport of the pesticide under review by wind erosion, the risk assessments typically use average soil losses ranging from 1 to 10 tons/ha·year, with a typical value of 5 tons/ha·year. The value of 5 tons/ha·year is equivalent to 500 g/m² (1 ton=1000 kg and 1 ha = 10,000 m²) or 0.05 g/cm² (1m²=10,000 cm²). Using a soil density of 2 g/cm³, the depth of soil removed from the surface per year would be 0.025 cm [(0.05 g/cm²)÷ (2 g/cm³)]. The average amount per day would be about 0.00007 cm/day (0.025 cm per year ÷ 365 days/year). This central estimate is based on a typical soil loss rate of 5 tons/ha·year. Since the range of plausible rates of annual soil loss is 1 to 10 tons/ha·year, the range of soil loss per day may be calculated as 0.00001 cm/day (0.00007 ÷ 5 = 0.000014) to 0.0001 cm/day (0.00007×2 = 0.00014).

The amount of the pesticide that might be transported by wind erosion depends on several factors, including the application, the depth of incorporation into the soil, the persistence in the soil, the wind speed, and the topographical and surface conditions of the soil. Under desirable conditions, like relatively deep (10 cm) soil incorporation, low wind speed, and surface conditions that inhibit wind erosion, it is likely that wind transport of herbicides would be neither substantial nor significant.

As with the deposition of the pesticide in runoff, the deposition of the pesticide in contaminated soil from wind erosion will vary substantially with local conditions. For most risk assessments, neither concentration nor dispersion is considered quantitatively. Nonetheless, these factors together with the general and substantial uncertainties in the exposure assessment are considered in the risk characterization (Section 4.4).

4.2.3.6. Volatilization – For some herbicides, off-site volatilization may be an important route of exposure for nontarget species. General methods for estimating exposures from volatilization have not been developed. Thus, this section is included only when the chemical-specific information is adequate to support both an exposure assessment and a dose-response assessment. This is consistent with the basic approach to risk assessment discussed in Section 1.2.1.

4.2.4. Soil Organisms

For soil organisms, the toxicity data are usually expressed in units of soil concentration – i.e., mg pesticide/kg soil. These units are equivalent to parts per million (ppm) concentration in soil. The GLEAMS modeling discussed in Section 3.2.3.4 provides estimates of concentration in soil as well as estimates of off-site movement (losses in runoff, sediment, and by percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are estimated.

4.2.5. Aquatic Organisms

In virtually all of the Forest Service risk assessments, the exposure assessment for aquatic species is identical to the estimated peak and longer-term concentrations in water that are used in the human health risk assessment as well as in the exposure assessments for terrestrial wildlife from the consumption of contaminated water. Some elaboration of these exposure assessments may be provided for pesticides that are intended for direct application to water. These are detailed and discussed on a case-by-case basis.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The dose-response assessment for ecological effects can range from very simple to extremely elaborate depending on the amount and type of information that is available, as well as levels of exposure that are plausible. Dose-response assessments are typically presented for each of the major groups of nontarget organisms on which data are available. These groups are identified in the Hazard Identification (Section 4.1) and often include terrestrial mammals, birds, soil organisms, microorganisms, terrestrial plants, and aquatic species (fish, aquatic invertebrates, and aquatic plants). Sub-groupings may be given (e.g., warm water and cold water fish) if sufficient data are available.

As a general rule, the dose-response assessment is kept as simple as possible. For example, if the plausible level of exposure for the most sensitive species is below the level of concern (defined in Section 4.4.), the dose-response assessment may be presented as a point estimate – i.e., a single value, most often an NOEL or NOEC. Point estimates are also used when this is the only type of estimate supported by the available data. In most cases, however, an attempt is made to differentiate sensitive and tolerant species for each group of organism. This is consistent with the extreme values approach currently used in Forest Service risk assessments (Section 1.2.2.2). Whenever possible and appropriate, both acute and chronic NOEL or NOEC values are given for both sensitive and tolerant species. In some instances, acute toxicity values may be available for both sensitive and tolerant species but chronic toxicity values are available for only one group – i.e., sensitive or tolerant species. In such cases, the relative potency method may be used to estimate a chronic value for the group on which experimental data are missing. In some instances, a large amount of toxicity data may be available on a large number of species within a particular group. In such cases, more elaborate methods may be used to quantify risks for various species within the group.

4.3.2. Point Estimates

As the name implies, point estimates are simply single numbers. In terms of the dose-response assessment, these values may be NOEC, NOEL, LD_x , or EC_x values where LD_x and EC_x designate a lethal dose (LD) or effective dose (ED) at some designated response rate – e.g., LD_{50} for a dose that is estimated to be lethal to 50% of a population. As a matter of policy, the Forest Service prefers to use NOEC or NOEL values rather than LD_x or EC_x values. In some cases, however, only LD_x or EC_x values may be available and these will be used. The consequences of using LD_x or EC_x values are then discussed in the risk characterization (Section 4.4).

4.3.3. Extreme Values

As discussed in Section 1.2.2.2, Forest Service risk assessments are currently based on an extreme values approach. Almost no values used in a risk estimate – either toxicity values or exposure values – are presented as a single number. As detailed in Section 3.2 and 4.2, most exposure assessments are presented as central estimates with an upper and lower bound. Whenever possible, toxicity values are presented for *sensitive* and *tolerant* species. The value for sensitive species is typically the lowest reported experimental value that is available, and the

value for tolerant species is the highest reported experimental value. In some cases, considerations of data quality, data documentation, and the geographical distribution of the species may be used to censor some data from the selection of the sensitive and tolerant species. For example, take a report in a secondary source indicating that a particular species of fish native to Southeast Asia is either remarkably more or less sensitive to a particular pesticide than fish native to North America. In most cases, the data on North American fish will come from studies submitted to U.S. EPA that are very well documented or studies from the peer-reviewed open literature. In such a case, the less well documented information on the extremely sensitive or tolerant fish from Southeast Asia might not be used. These sorts of judgements are considered on a case-by-case basis in the dose-response assessment for each group of organisms considered in the risk assessment. As with the point estimate approach, NOEC or NOEL values are used, when available, rather than LD_x , or EC_x values. If LD_x , or EC_x values must be used, LD_5 , or EC_5 values are preferred over LD_{50} or EC_{50} values.

4.3.4. Relative Potency Method

In some instances, acute toxicity values may be available for both sensitive and tolerant species but chronic toxicity values are available for only one group – i.e., sensitive or tolerant species. In such cases, the relative potency method may be used to estimate a chronic value for the group on which experimental data are missing. This method can be used for NOEC, LD_x , or analogous values. Defining the acute toxicity (AT) value for the sensitive species as AT_s , the acute toxicity value for the tolerant species as AT_T , and the known chronic toxicity (CT) value for the tolerant species as CT_T , the chronic toxicity value for the sensitive species (CT_s) is estimated as:

$$CT_s = CT_T \times AT_s / AT_T \quad (\text{Eq. 4-1a})$$

Alternatively, the chronic toxicity value for the tolerant species (CT_T) might be estimated from the chronic toxicity value for the sensitive species (CT_s) as:

$$CT_T = CT_s \times AT_T / AT_s \quad (\text{Eq. 4-1b})$$

This approximation is discussed and justified on a case-by-case basis.

4.3.5. Other Methods

More elaborate methods of characterizing dose-response assessments may be used if justified by the nature of the available data. In the context of probabilistic risk assessments, several methods are available for assessing variability among species based on species sensitivity distributions (e.g., Posthuma et al. 2002). As noted in Section 1.2.2, the Forest Service has not adopted full probabilistic methods at this time. Nonetheless, the same basic concepts have been applied when the data have been available and the potential risks to nontarget species appeared to justify the elaboration.

An example of one such approach is illustrated in Figure 4-1, which summarizes the dose-response assessment for non-target terrestrial invertebrates exposed to *Bacillus thuringiensis* var.

kurstaki (*B.t.k.*). For *B.t.k.*, the sensitive species are all lepidoptera, and all of the studies used in the analysis involve feeding various lepidopteran larvae with vegetation treated with various *B.t.k.* formulations at rates that can be expressed in units of BIU/ha. Data were available on seven species of lepidoptera: two target species (the gypsy moth and cabbage looper) and five non-target species (the Karner blue butterfly, two species of swallowtail butterfly, the promethea moth, and late instars of the cinnabar moth). The data on tolerant species used in the dose-response assessment involved feeding of early instar cinnabar moth larvae as well as direct spray of non-lepidopteran insects: green lacewing adults as well as larvae and direct spray of adult lady beetles.

The analysis derives dose-response relationships for both sensitive and insensitive species—i.e., estimates of mortality were based on the application rate. Sensitive species have an LD₅₀ value of about 21 BIU/ha and consist entirely of lepidoptera. The tolerant species have an LD₅₀ of about 590 BIU/ha, which is approximately 28 times greater than the LD₅₀ value for sensitive species.

For statistical analysis, the probit model (Section 3.3.4.) was used. Because different studies are combined, each with different control response rates, standard probit analysis was not used. Instead, the responses attributable to *B.t.k.* based on Abbott's formula were converted to probits using the inverse normal function in EXCEL:

$$\text{Probit} = 5 + \text{NORMINV}(P, 0, 1)$$

where 0 and 1 are the mean and standard deviation of the standard normal curve, and *P* is as defined above. *[Note: The equations used in the example in this section are not numbered. This is intentional because these equations are specific to this example and will not be cited or referred to further.]*

The constant of 5 is the standard constant for converting normal equivalent deviates to probits. Thus, a probit of 5 represents a response of 50%, a probit of 6 represents a response that is one standard deviation above 50% (i.e., a response of about 82%), a probit of 7 represents a response that is two standard deviations above 50% (i.e., a response of about 98%) and so on.

Using this transformation, the probit responses (independent variable) and log₁₀ BIU/acre were used to estimate the linearized dose-response function:

$$Y = a + bx$$

using standard linear regression where *Y* is the probit response, *x* is the log₁₀ of the BIU/acre treatment, *b* is the slope of the dose-response curve, and *a* is the intercept.

The log-dose probit-response model provided a statistically significant fit to data for the sensitive ($p \approx 0.0004$, adjusted $r^2 = 0.79$) and the tolerant ($p \approx 0.00003$, adjusted $r^2 = 0.95$) species. In

addition, the slopes of the dose-response curves are similar and not significantly different—1.95 with a 95% confidence interval of about 1.2 to 2.7 for sensitive species, and 2.6 with a 95% confidence interval of about 2.1 to 3.2 for tolerant species.

Consequently, the regression analysis was run a second time using a variable, S , assigned a value of 1 for sensitive species and 0 for tolerant species in order to constrain the slopes of the two curves to be equal:

$$Y = a + bx + cS$$

where c is the coefficient for the sensitivity variable, S , and the other terms are as defined above.

The data on both sensitive and tolerant species combined fits the following model:

$$Y = -1.48 + 2.34 x + 3.36 S$$

with a highly significant p -value (8.4×10^{-11}) and an adjusted r^2 of about 0.95—i.e., the model explains 95% of the variability in the data, and the probability that the association occurred by random chance is about 1 in 11 billion. It is worth noting that the p -value for the variable for sensitivity is about 2.8×10^{-11} , indicating a highly significant difference between the sensitive and tolerant species—i.e., the probability that the apparent difference occurred by chance is about 1 in 36 billion. Based on this analysis, the relative potency of *B.t.k.* to sensitive species is about 28, relative to tolerant species [$590 \text{ BIU/ha} \div 21 \text{ BIU/ha}$].

Again, analyses such as these are both data intensive and labor intensive and are not conducted unless the risk assessment suggests that nontarget species are likely to be at risk, and thus the dose-response assessment must be elaborated as fully as possible in order to provide the fullest possible characterization of risk.

4.4. RISK CHARACTERIZATION

The risk characterization for ecological risk assessments is mathematically similar to the HQ approach discussed for the human health risk assessment (Section 3.4). Consequently, the reader is referred to Section 3.4 for a discussion of hazard quotients.

Conceptually, however, there is one substantial difference between the risk characterization for the human health risk assessment and the ecological risk assessment: the level of tolerable risk. In human health risk assessments, the fundamental concern is with the individual. RfD values and other similar estimates are intended to represent population thresholds. Thus, if the level of exposure is below the RfD – i.e., the HQ is less than unity – no effects are anticipated in any individuals. In ecological risk assessment, concern is most often with populations of animals rather than individual animals. Thus, no attempt is made to derive RfD-like estimates with the application of uncertainty factors.

This general approach to risk characterization is conceptually similar to that taken by the U.S. EPA's Environmental Fate and Effects Division (EFED) of the Office of Pesticide Programs (U.S. EPA/EFED 2004). One very superficial difference involves nomenclature. EFED uses the term **RQ** (risk quotient) rather than HQ (hazard quotient). This is purely a difference in nomenclature and the two terms represent the same thing – i.e., a level of exposure divided by some measure of a toxic or nontoxic dose.

The approach used by the U.S. EPA is summarized in Table 4-2. Note that the term EEC used in Table 4-2 represents the estimated environmental concentration. This corresponds to the exposure assessments discussed in Section 4.2. As indicated in Table 4-2, the U.S. EPA/EFED (2004) defines the level of concern somewhat differently for each group of organisms and the level of concern is varied based on differences in the endpoint (e.g., NOEC or EC_x) that is used. This approach also explicitly considers differences in ecological status of the organism by using different levels of concern for endangered species.

As detailed in U.S. EPA/EFED (2004), the U.S. EPA risk assessments conducted by EFED (i.e., within the Office of Pesticide Programs) are part of the pesticide registration process and the specific LOCs detailed in Table 4-2 are considered as screening tools. If a particular risk assessment results in an HQ that exceeds the LOC, additional analyses may be conducted and may involve elaboration or refinement of the dose-response relationships or exposure assessments. This approach reflects the U.S. EPA's interpretation and implementation of its legislative mandates under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA), both amended by the Food Quality Protection Act (FQPA) of 1996.

The risk assessments conducted by or for the USDA Forest Service are in response to the National Environmental Policy Act (NEPA). In implementing the NEPA process, the generic or programmatic risk assessments detailed in this document are only the initial step in the risk assessment process and are analogous (although not identical) to what the U.S. EPA refers to as a

screening level risk assessment (U.S. EPA/EFED 2004, p. 31 ff). Some differences between the EPA screening level risk assessments and generic risk assessments conducted for the Forest Service involve the use of extreme values rather than simply the most conservative value. As detailed in Section 4.3.3, the Forest Service risk assessments do not focus solely on the most sensitive species (as in most U.S. EPA risk assessments) but also consider a range of sensitivities within groups of organisms when such information is available. Similar differences exist in the use of extreme values in the exposure assessments (Section 4.2).

As noted above, the generic risk assessments prepared in support of Forest Service programs are only the first step in a much broader process. Typically, before the Forest Service will conduct or support any pesticide application, a programmatic Environmental Impact Statement (EIS) will be prepared that specifically addresses the issues associated with a programmatic goal (e.g., the control of a specific pest or class of pests) in a specific region. Subsequent to the EIS and prior to a pesticide application, an additional site-specific assessment, referred to as an Environmental Assessment (EA) may be conducted. It is during the preparation of an EIS and/or an EA that specific consideration is given to threatened and endangered species, steps that can be taken to mitigate risks, as well as a number of other regional or site-specific conditions that could impact the risk characterization.

Because of the differences in the use of U.S. EPA and Forest Service risk assessments, the system used by U.S. EPA (as summarized in Table 4-2) is not adopted directly. The alternative approach used in Forest Service risk assessments is summarized in Table 4-3. Consistent with the above discussion, the generic Forest Service risk assessments will not typically develop a separate risk characterization for threatened and endangered species. As noted in Section 4.3, the extreme value method will consider variability in all species within a group and will identify information on threatened and endangered species when such information is available. This information may then be used in an EIS or EA.

Another difference involves the use of toxicologic endpoints and the related issue of LOC values. As summarized in Table 4-2, the U.S. EPA will use fractions of the LD_{50} or LC_{50} values with different LOCs for different groups. The justification for these differences in the context of the goals of the U.S. EPA are discussed in the EPA's methodology document (U.S. EPA/EFED 2004). Historically, the Forest Service and their collaborators have preferred to use NOAEL or NOEC values rather than the LD_{50} or LC_{50} values as the denominator in risk quotients. In addition, the Forest Service prefers to use a consistent LOC of 1 for the interpretation of all HQ values.

In some cases, however, adopting an approach based on LD_{50} or LC_{50} values analogous to that of the U.S. EPA may be more prudent and protective. For example, the U.S. EPA uses a LOC of 0.1 with an LC_{50} value for threatened and endangered species of aquatic animals (Table 4-2). This is equivalent to using the LC_{50} value divided by 10 with an LOC of 1. This approach may sometimes yield an estimate that is lower than the NOEC value. This can occur because of the apparent slope of the dose-response relationship (Eq. 3-17 in Section 3.3.4), the concentration or

dose spacing used in the bioassay, and the number of organisms in each exposure group. This situation would occur less often, of course, with an LOC of 0.5, the LOC used by U.S. EPA for acute risk.

While the variability of LOC values as well as the variability of endpoints for different groups of organisms appear to meet the needs of U.S. EPA, this type of variability does not meet the needs of the Forest Service. Thus, as summarized in Table 4-3, the Forest Service risk assessments will examine the dose-response relationships and will select either an NOEC value. If an NOEC value is not available, a median lethal or effective exposure value divided by 10 will be used. In either case, an LOC of 1 is used for the interpretation of HQ values. This is consistent with the approach used in the human health risk assessment (Section 3.4) and is also conceptually consistent with the approach used by U.S. EPA.

Notwithstanding the general use of this HQ based approach, some Forest Service risk assessments may use more elaborate dose-response assessments (Sections 3.3 and 4.3) based either on field studies or quantitative dose-response curves. This is similar to methods that are used by U.S. EPA when their screening level risk assessments trigger a more detailed analysis (U.S. EPA/EFED 2004). In such cases, the HQ approach in the risk characterization may be supplemented with discussions concerning the probability, severity, and/or duration of some adverse effect.

5. REFERENCES

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FIGURES

- Figure 1-1:** Overview of Risk Assessment process
- Figure 1-2:** Example of Monte Carlo Analysis
- Figure 3-1:** Schematic Overview of Dermal Absorption Processes
- Figure 3-2:** Composition (%) of the blood and skin (data from ICRP 1992 and Klein-Szanto et al. 1991)
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- Figure 4-1:** Elaborated dose-response assessment for nontarget terrestrial invertebrates exposed to *Bacillus thuringiensis* var. *kurstaki*.

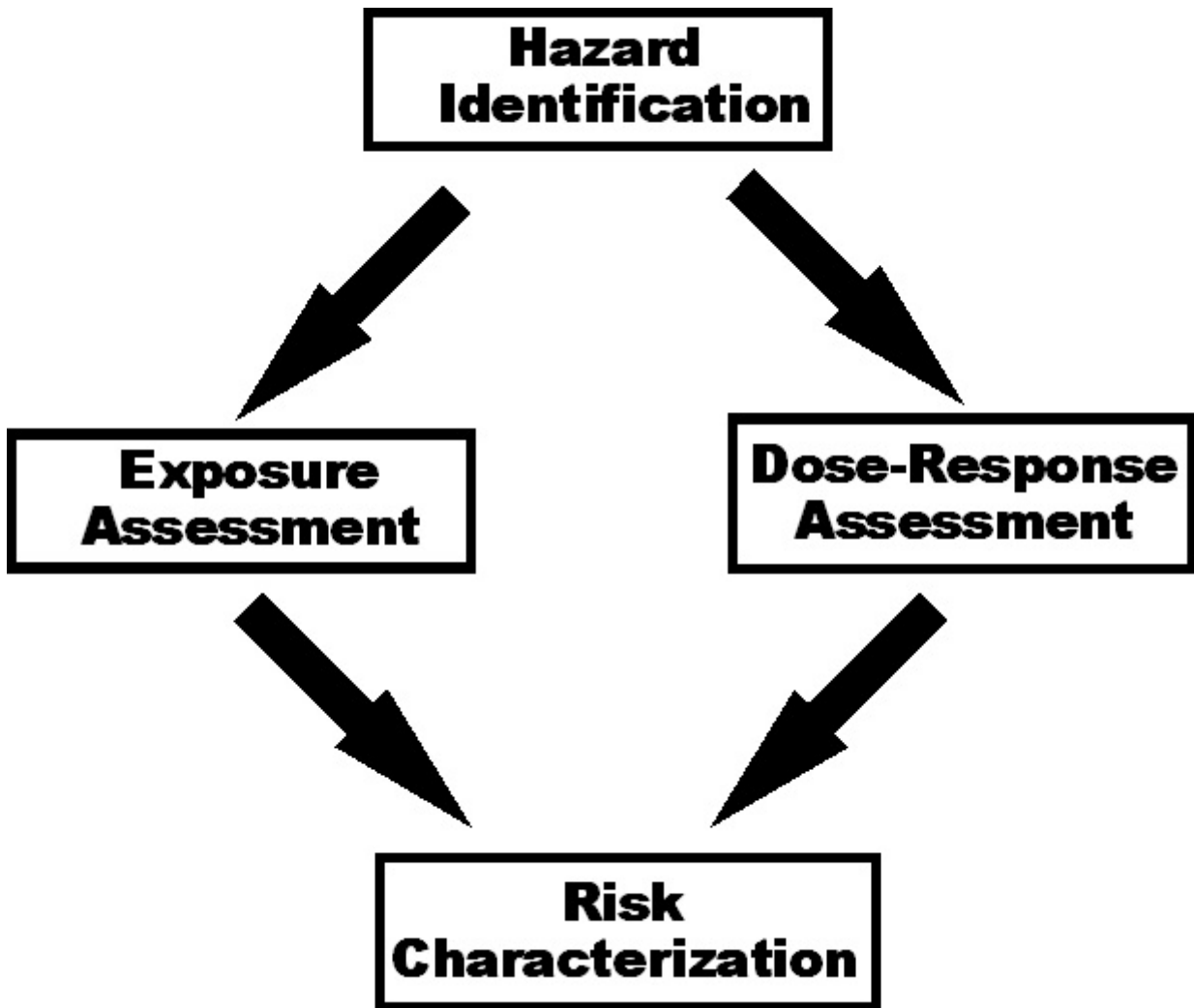


Figure 1-1: Overview of risk assessment process.

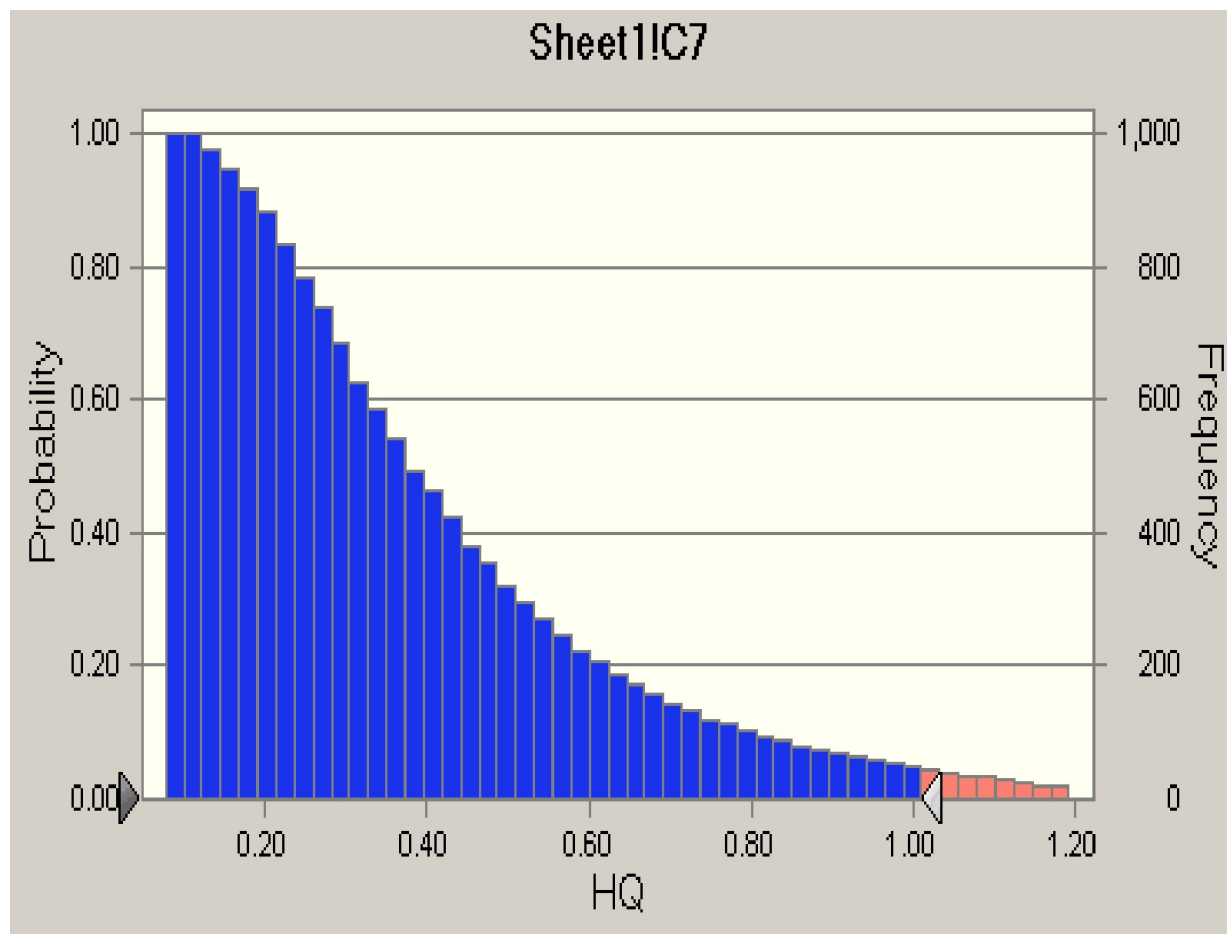


Figure 1-2: Example of Monte Carlo Analysis. See Section 1.2.2.1.

HQ = (Exposure/Body Weight)/RfD	
Parameter	Distribution
Body weight	Normal distribution with a mean of 70 kg and a standard deviation of 10 kg
Exposure	Uniform distribution with a range of 50 mg/day to 200 mg/day.
RfD	Triangular with a mode of 3.5 mg/kg/day, a lower limit of 2 mg/kg/day and an upper limit of 10 mg/kg/day

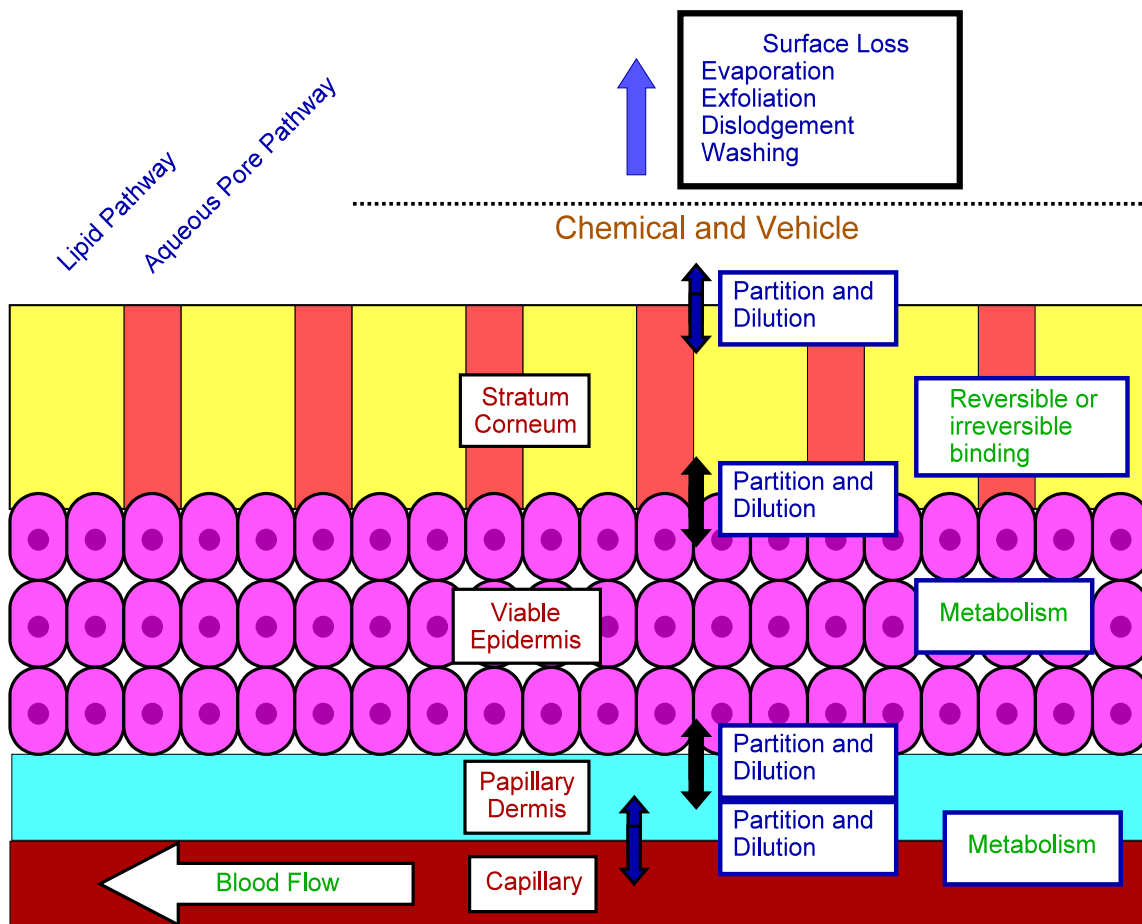


Figure 3-1: Schematic Overview of Dermal Absorption Processes (modified from U.S. EPA 1992 and Flynn 1990).

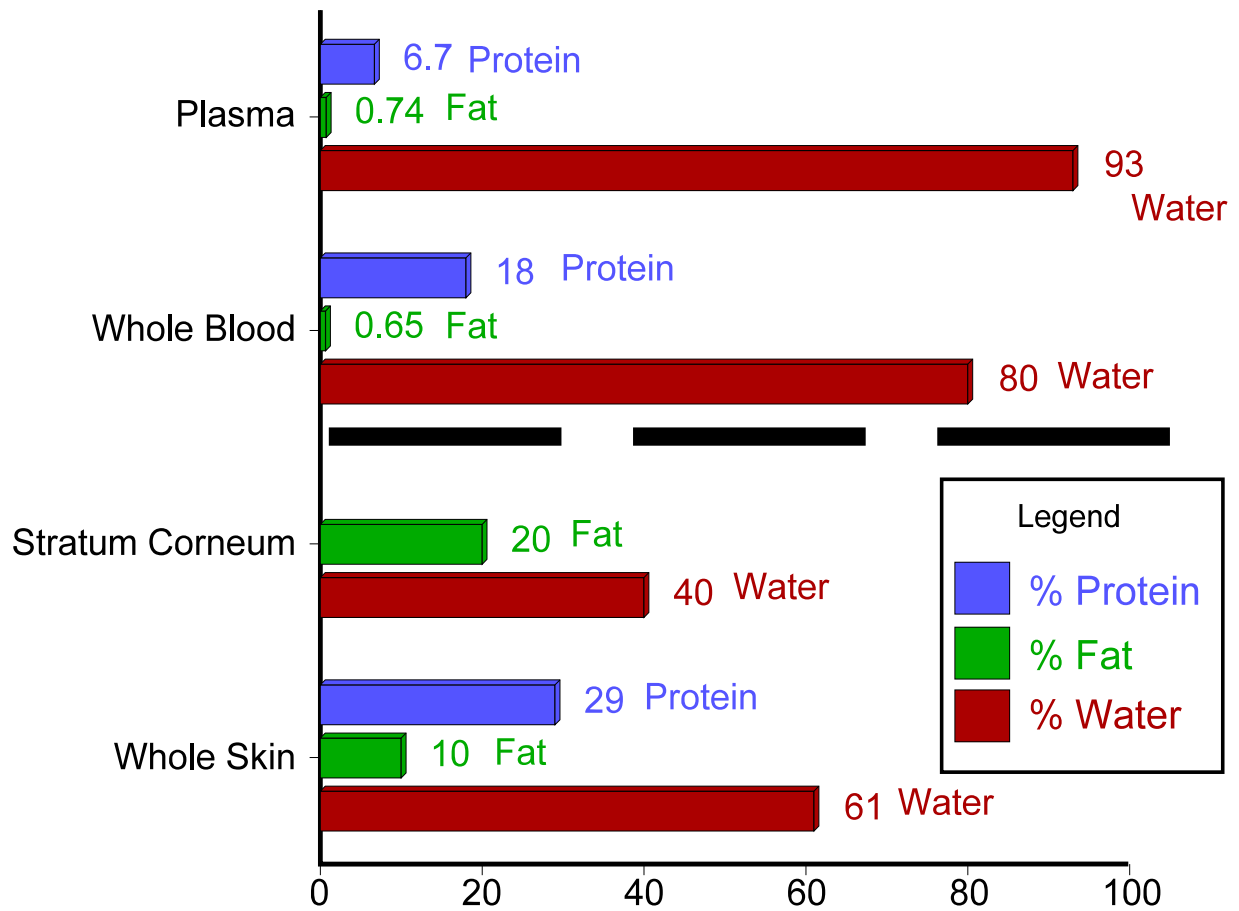


Figure 3-2: Composition (%) of the blood and skin (data from ICRP 1992 and Klein-Szanto et al. 1991).

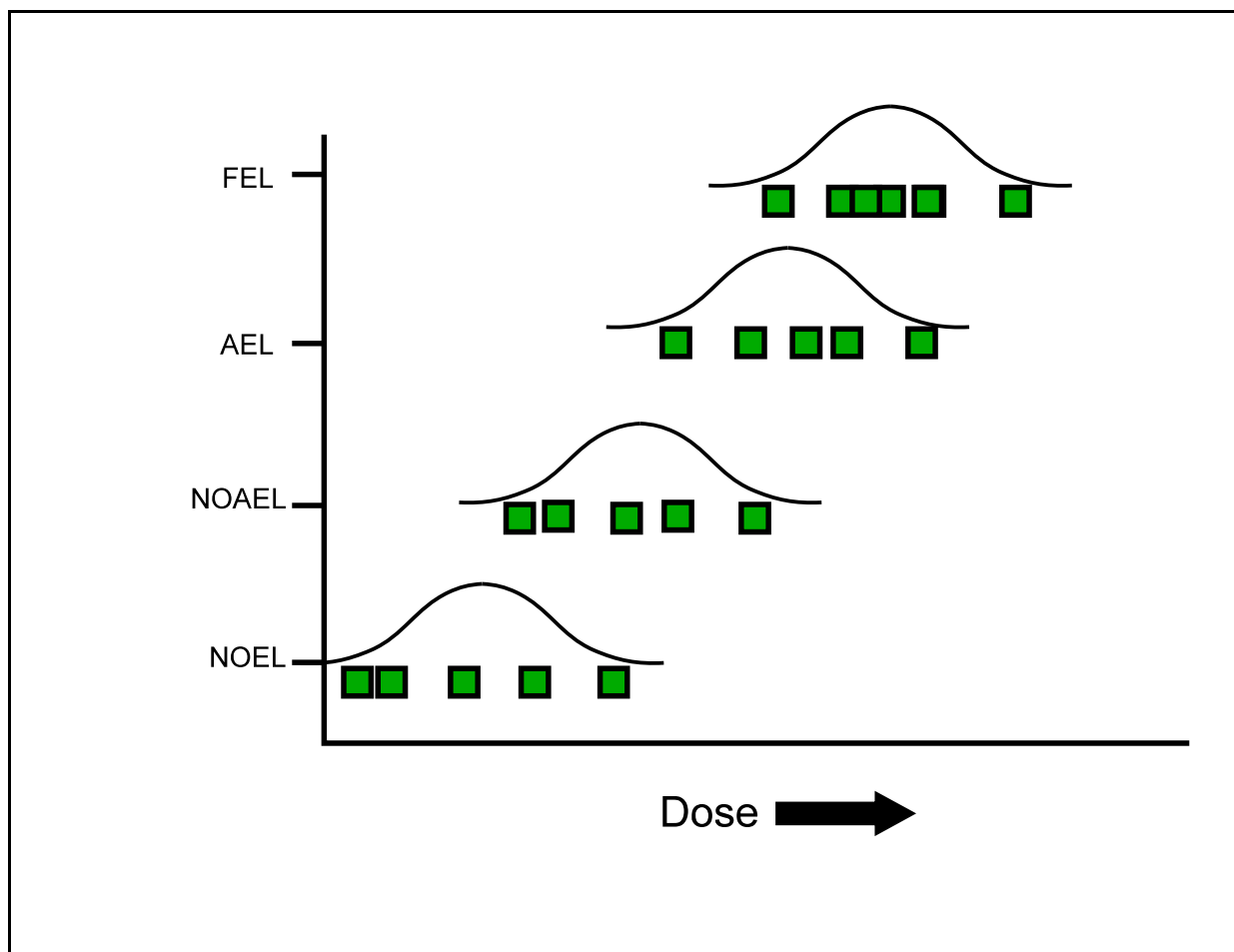


Figure 3-3: Conceptual overview of categorical regression.

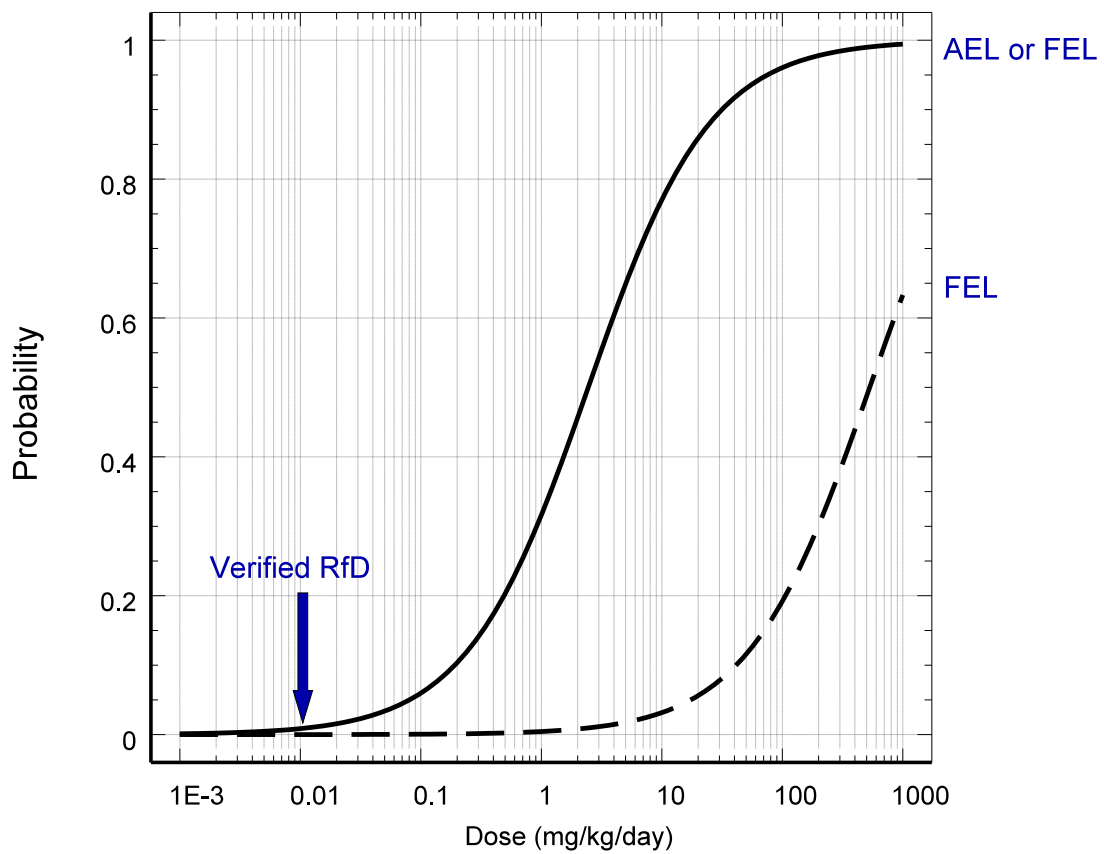


Figure 3-4: Example of categorical regression applied to data on 2,4-D.

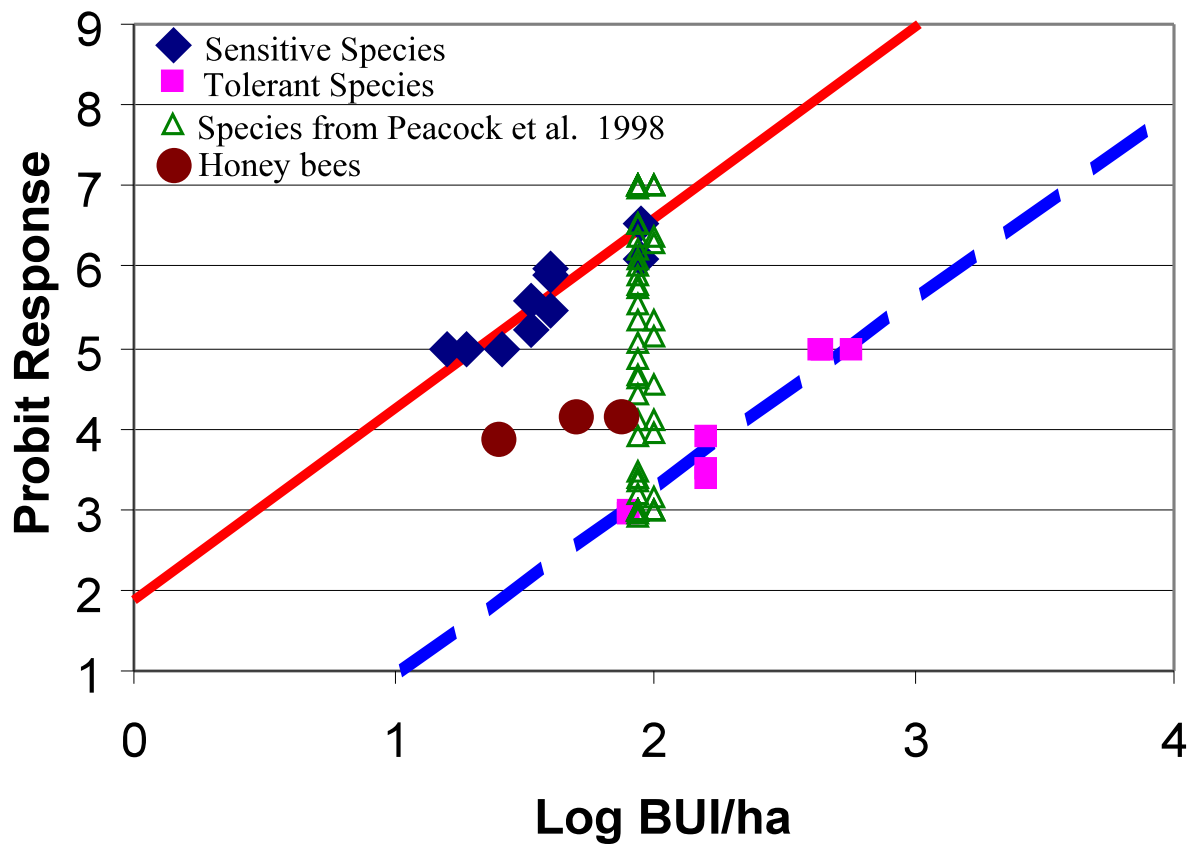


Figure 4-1: Elaborated dose-response assessment for nontarget terrestrial invertebrates exposed to *Bacillus thuringiensis* var. *kurstaki*. Taken from SERA TR 03-43-05-02c dated June 8, 2004.

TABLES

Table 1-1: Severity definitions used in human health risk assessment (HHRA) and ecological risk assessment (ERA)

Table 3-1: Comparison of dermal absorption and estimated dermal permeability of hydrocortisone and testosterone with some of their esters

Table 3-2: Toxicity categories used by the U.S. EPA for pesticide labeling and classifications in human health risk assessment

Table 3-3: Occupational exposure rates used in risk assessments

Table 3-4: Dose-response assessments conducted by the federal government and related organizations

Table 3-5: Uncertainty factors used to derive reference values

Table 3-6: Qualitative Summary of dose-severity relationships for 2,4-D.

Table 4-1: Toxicity categories used in ecological risk assessments

Table 4-2: Level of concern (LOC) by risk presumption category

Table 1-1: Severity definitions used in human health risk assessment (HHRA) and ecological risk assessment (ERA)

Acronym		Definition
HHRA	ERA	
NOEL	NOEC	<i>No-observed-effect level (concentration):</i> No biologically or statistically significant effects attributable to treatment.
NOAEL	NOAEC	<i>No-observed-adverse-effect level (concentration):</i> Effects that are attributable to treatment but do not appear to impair the organism's ability to function and clearly do not lead to such an impairment.
LOEL	LOEC	<i>Lowest-observed-effect level (concentration):</i> The lowest exposure level associated with an adverse effect.
AEL		<i>Adverse-effect level:</i> Signs of toxicity that must be detected by invasive methods, external monitoring devices, or prolonged systematic observations. Symptoms that are not accompanied by grossly observable signs of toxicity.
FEL		<i>Frank-effect level:</i> Gross and immediately observable signs of toxicity.

Table 3-1: Comparison of dermal absorption and estimated dermal permeability of hydrocortisone and testosterone with some of their esters

Chemical	MW ^a	log K _{ow} ^a	K _p ^b	% Absorption ^c
Hydrocortisone	362.47	1.61	0.00016	1.87
Hydrocortisone acetate	404.51	2.30	0.00028	2.55
Testosterone	288.43	3.32	0.0075	13.24
Testosterone acetate	330.47	4.27	0.020 [0.032]	4.62
Testosterone propionate	344.50	4.77	0.037 [0.032]	3.34

^a Durkin et al. (1995)

^b Calculated using Equation 3-1. Limits based on Flynn (1990) in brackets. See text for details.

^c Feldmann and Maibach (1969). Cumulative percent absorption over a 5-day observation period.

Table 3-2: Toxicity categories used by the U.S. EPA for pesticide labeling and classifications in human health risk assessment (U.S. EPA/OPP 2003)

Type of test	Category			
	I	II	III	IV
Oral LD ₅₀	Up to and including 50 mg/kg	From 50 to 500 mg/kg	From 500 to 5000 mg/kg	Greater than 5000 mg/kg
Inhalation LC ₅₀	Up to and including 0.2 mg/liter	From 0.2 to 2 mg/liter	From 2 to 20 mg/liter	Greater than 20 mg/liter
Dermal LD ₅₀	Up to and including 200 mg/kg	From 200 mg/kg thru 2000 mg/kg	From 2000 mg/kg thru 20,000 mg/kg	Greater than 20,000 mg/kg
Eye effects	Corrosive; corneal opacity not reversible within 7 days	Corneal opacity reversible within 7 days; irritation persisting for 7 days.	No corneal opacity; irritation reversible within 7 days	No irritation
Skin effects	Corrosive	Severe irritation at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours
Definitions of Categories				
I	All pesticide products meeting the criteria of Toxicity Category I shall bear on the front panel the signal word <i>Danger</i> . In addition if the product was assigned to Toxicity Category I on the basis of its oral, inhalation or dermal toxicity (as distinct from skin and eye local effects) the word <i>Poison</i> shall appear in red on a background of distinctly contrasting color and the skull and crossbones shall appear in immediate proximity to the word <i>poison</i> .			
II	All pesticide products meeting the criteria of Toxicity Category II shall bear on the front panel the signal word <i>Warning</i> .			
III	All pesticide products meeting the criteria of Toxicity Category III shall bear on the front panel the signal word <i>Caution</i> .			
IV	All pesticide products meeting the criteria of Toxicity Category IV shall bear on the front panel the signal word <i>Caution</i> .			

Table 3-3: Occupational exposure rates used in risk assessments

Worker Group	Rate (mg/kg bw/day per lb applied)		
	Central	Lower	Upper
Directed foliar	0.003	0.0003	0.01
Broadcast foliar	0.0002	0.00001	0.0009
Aerial	0.00003	0.000001	0.0001

^a See Section 3.2.2.1 for a detailed discussion of the data on which these estimates are based.

Table 3-4: Dose-response assessments conducted by the federal government and related organizations

Acronym	Definition	Reference
Systemic Toxicity (Non-carcinogenic)		
RfD	<i>Reference Dose:</i> Oral dose (mg/kg/day) not likely to be associated with adverse effects over lifetime exposure, in the general population, including sensitive subgroups.	U.S. EPA 1987
RfD _s	<i>Subchronic Reference Dose:</i> Oral dose (mg/kg/day) not likely to be associated with adverse effects over a less-than-lifetime exposure, in the general population, including sensitive subgroups. [The exposure duration to which this value applies is not clearly defined.]	U.S. EPA 1990
RfD _{rt}	<i>Reference Dose for Reproductive Toxicity:</i> Oral dose (mg/kg/day) not likely to be associated with adverse developmental effects, in the general population, including sensitive subgroups. Used to evaluate effects after single exposure episode.	U.S. EPA 1991a
RfC	<i>Reference Concentration:</i> Concentration in air (mg/m ³) not likely to be associated with adverse effects over lifetime exposure, in the general population, including sensitive subgroups.	U.S. EPA 1990
MRL	<i>Minimal Risk Level:</i> A route-specific (oral or inhalation) and duration- specific estimate of an exposure level that is not likely to be associated with adverse effects in the general population, including sensitive subgroups.	ATSDR 1992
1-Day HA	<i>1-Day Health Advisory:</i> A drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, after 1-day of exposure.	U.S. EPA 1989c
10-Day HA	<i>10-Day Health Advisory:</i> A drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, over a 10-day exposure period.	U.S. EPA/ODW 1990
TLV	<i>Threshold Limit Value:</i> An air concentration (mg/m ³) not likely to cause adverse effects in exposed workers, over a normal period of work.	ACGIH 1992
Carcinogenicity		
Slope Factor [q ₁ *]	<i>Cancer Potency Parameter:</i> A model-dependent measure of cancer potency (mg/kg-day) ⁻¹ over lifetime exposure. [Often expressed as a q ₁ * which is the upper 95% confidence limit of the first dose coefficient (q ₁) from the multistage model.]	U.S. EPA 1996b,2003
Unit Risk _{air}	<i>Unit Risk for Inhalation Exposures:</i> The risk associated with a continuous lifetime exposure to an air concentration expressed (mg/m ³) ⁻¹ or (µg/m ³) ⁻¹ .	U.S. EPA 1996b,2003
Unit Risk _{water}	<i>Unit Risk for Water Consumption:</i> The risk associated with a continuous lifetime exposure to a drinking water concentration expressed (mg/L) ⁻¹ or (µg/L) ⁻¹ .	U.S. EPA 1996b,2003

Table 3-5: Uncertainty factors used to derive reference values*

Definitions			
Factor	Basis	ATSDR	U.S. EPA
Interhuman	Use a 10-fold factor when extrapolating from valid experimental results using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among humans.	yes	yes
Experimental to human	Use a 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies on human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to humans. If methods are available for a more explicit extrapolation, this uncertainty factor can be reduced or eliminated.	yes	yes
LOAEL to NOAEL	Generally use a 10-fold factor when deriving a reference value, RfD, or MRL from a LOAEL instead of an NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs.	yes - UF always 10	yes -UF varies
Subchronic to chronic	Generally use a 10-fold factor when deriving a reference value or RfD from less than chronic results on experimental animals or humans. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs.	no	yes
Children	An additional uncertainty factor of 10 is required for the protection of children unless the available data indicate that this factor is not required.	no	yes
Incomplete database	Generally use a 10-fold factor when deriving a reference value or RfD from valid results in experimental animals when the data are "incomplete." This factor is intended to account for the inability of any study to address all possible adverse outcomes.	no	yes
Modifying factor	Use professional judgment to determine an additional uncertainty factor that is >1 and ≤ 10 for deriving a reference value or RfD. The magnitude of the MF depends upon the professional assessment of the scientific uncertainties of the study and database not explicitly treated above. The default for the MF is 1	no	yes

* Adapted from ATSDR 2004a; U.S. EPA 1980, 1987; U.S. EPA/OERD. 2000, U.S. EPA/OPP 1998

Table 3-6: Qualitative Summary of dose-severity relationships for 2,4-D.

Animal Dose	Estimated Human Dose	Effect
<0.01-0.1	<0.001-0.01	No effects are likely.
0.1-1	0.01-0.1	At the upper end of the range, a slight increase in thyroid weight and/or decrease in testicular weight may be noted. Possible decrease in whole body weight gain.
1-10	0.1-1	In addition to above, subclinical signs of neurologic toxicity are possible. Subclinical pathology to the kidney and liver.
10-100	1-10	Subclinical signs of neurologic toxicity are likely and mild signs of toxicity are plausible (60 mg/kg/day). Degenerative or other pathological changes to several organs are likely. Upper limit of the range may be lethal.
100-1,000	10-100	Frank neurological and/or reproductive effects, including terata are likely. Upper limit of the range will be lethal without prompt and effective medical intervention.

All doses in units of mg a.e./kg bw/day.

Table 4-1: Toxicity categories used in ecological risk assessments (U.S. EPA/EFED 2001)

Category	Type of Test			
	Avian oral	Avian dietary	Aquatic	Wild mammals oral
Very highly toxic	Less than 10 mg/kg	Less than 50 ppm	Less than 0.1 mg/L	Less than 10 mg/kg
Highly toxic	10 to 50 mg/kg	50 to 500 ppm	0.1 to 1 mg/L	10 to 50 mg/kg
Moderately toxic	51 to 500 mg/kg	501 to 1000 ppm	Greater than 1 to 10 mg/L	51 to 500 mg/kg
Slightly toxic	501 to 2000 mg/kg	1001 to 5000 ppm	Greater than 10 to 100 mg/L	501 to 2000 mg/kg
Practically nontoxic	Greater than 2000 mg/kg	Greater than 5000 ppm	Greater than 100 mg/L	Greater than 2000 mg/kg

Table 4-2: Level of concern (LOC) by risk presumption category (U.S. EPA/EFED 2004).

Risk Presumption	RQ	LOC
Mammals and Birds		
Acute Risk ^a	EEC ^b /LC ₅₀ or LD ₅₀ /sqft ^c or LD ₅₀ /day ^d	0.5
Acute Restricted Use ^e	EEC/LC ₅₀ or LD ₅₀ /sqft or LD ₅₀ /day (or LD ₅₀ <50 mg/kg)	0.2
Acute Endangered Species ^f	EEC/LC ₅₀ or LD ₅₀ /sqft or LD ₅₀ /day	0.1
Chronic Risk	EEC/NOEC	1
Aquatic Animals		
Acute Risk	EEC ^g /LC ₅₀ or EC ₅₀	0.5
Acute Restricted Use	EEC/LC ₅₀ or EC ₅₀	0.1
Acute Endangered Species	EEC/LC ₅₀ or EC ₅₀	0.05
Chronic Risk	EEC/NOEC	1
Terrestrial and Semi-aquatic Plants		
Acute Risk	EEC/EC ₂₅	1
Acute Endangered Species	EEC/EC ₀₅ or NOEC	1
Aquatic Plants		
Acute Risk	EEC ^h /EC ₅₀	1
Acute Endangered Species	EEC ^g /EC ₀₅ or NOEC	1

^a Potential for acute toxicity for receptor species if RQ > LOC (EPA, 2004).

^b Estimated environmental concentration (ppm) on avian/mammalian food items

^c mg/ft²

^d mg of toxicant consumed per day

^e Potential for acute toxicity for receptor species, even considering restricted use classification, if RQ > LOC (EPA, 2004).

^f Potential for acute toxicity for endangered species of receptor species if RQ > LOC (EPA, 2004).

^g EEC = ppb or ppm in water

^h EEC = lbs a.i./A

Table 4-3: Basis for Hazard Quotients in USDA/Forest Service Ecological Risk Assessments.

Duration	Basis for Hazard Quotient (HQ)	LOC
Mammals and Birds ^a		
Acute Risk	EEC/Acute NOEC EEC/(LD ₅₀ ÷ 10) – Used only if NOEC is unavailable or not defined.	1
Chronic Risk	EEC/NOEC Use most sensitive endpoint from reproduction study or field study.	1
Aquatic Animals ^b		
Acute Risk	EEC/Acute NOEC EEC/(LC ₅₀ ÷ 10) – Used only if NOEC is unavailable or not defined.	1
Chronic Risk	EEC/NOEC Full life-cycle study (preferred) or egg-and-fry study for fish.	1
Terrestrial and Semi-aquatic Plants ^b		
Risk	EEC/Acute NOEC EEC/(ED ₅₀ ÷ 10) – Used only if NOEC is unavailable or not defined.	1
Aquatic Plants ^b		
Algae	EEC/Acute NOEC EEC/(LC ₅₀ ÷ 10) – Used only if NOEC is unavailable or not defined.	1
Macrophytes	EEC/Acute NOEC EEC/(LC ₅₀ ÷ 10) – Used only if NOEC is unavailable or not defined.	1

^a The LD₅₀ values can be derived from either gavage or acute dietary exposures. Field studies may be used as alternatives but such studies are seldom adequately documented.

^b Field studies are sometimes available and may be preferable (i.e., more protective or more representative of programmatic uses) than laboratory bioassays.